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VOL. 31, SUPPLEMENTUM 112

THE DISTRIBUTION OF CATECHOL AMINES IN LOWER ANIMALS AND THEIR EFFECT ON THE HEART

BY

ERIC ÖSTLUND

STOCKHOLM 1954

ACTA PHYSIOLOGICA SCANDINAVICA
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From the Department of Physiology, Karolinska Institutet, Stockholm

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Printed in Sweden
APPELBERGS BOKTRYCKERI
UPPSALA 1954

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Preface

The investigation presented here was carried out at the Physiology Department I of the Karolinska Institute, Stockholm, to whose director, Professor U. S. von Euler, I am most grateful for his interest, advice and invaluable criticism throughout this work. I have pleasant memories of the period from 1942 to 1946, when I received my training in physiological research work under his supervision.

It is also a great pleasure to express my longstanding indebtedness to Docent E. Nyman, director of the IVth Medical Service of the St. Eriks Hospital, Stockholm, for his kindly interest and personal guidance.

My grateful acknowledgments are also due to:

Docents R. Fänge, H. Dunér, S. Gardell and Dr. P. Merton for their most appreciated advice and discussions when the work was being planned.

My colleagues and the staff of the Physiology Department for their kind help and interest during the course of this study.

Professor N. Holmgren and Dr G. Gustafson, Directors of the Marine Zoological Station at Kristineberg, and the members of their staff, for their kind interest during my work at Kristineberg, where part of the material was collected and prepared.

Miss I. Zimmerman for her help with the photographic work.

My wife, for her invaluable assistance and interest.

Mr S. H. Vernon for his translation from the Swedish.

The investigation was made possible by grants from the Therese and Johan Andersson Memorial Foundation, the Lars Hierta Memorial Fund, Karolinska Institute, and by a Government doctor's stipend.

Stockholm, March 1954. *Eric Östlund*

General Introduction

In recent years the rôle of noradrenaline as a transmitter of adrenergic nerve impulses and as an adrenal medullary hormone has been established (EULER, 1946 c, 1948 a; Holtz, Credner and Kroneberg, 1947). The occurrence, action and distribution of adrenaline and noradrenaline in the higher vertebrates have been the subject of numerous investigations reviewed by EULER (1950, 1951) and TAINTER and LUDUENA (1950).

The aim of the present investigation was to study the occurrence and distribution of adrenaline and noradrenaline in the lower animals and the effects of these and other catechol amines on the perfused hearts of some of the species.

Most invertebrates are found to contain very little if any adrenaline, noradrenaline or dopamine, except the insects. The relatively large amounts of noradrenaline and dopamine in insects have been briefly reported already (Östlund, 1953). In some invertebrates, however, and particularly in the lower vertebrates tested (fish) an unidentified substance was discovered which behaved chemically like a catechol amine and may be biologically active in these species.

The effect of catechol amines has been studied on the isolated hearts of various fishes (FÄNGE & ÖSTLUND, 1954). A summary of these experiments, together with the occurrence of catechol amines in the lower vertebrates, is given in Chapter V.

To facilitate a survey of the extensive literature, the latter has been divided in such a way that each chapter commences with a review of previous investigations in the field. A brief outline of the principal data on adrenaline and noradrenaline in the higher animals is given in the historical introduction.

CHAPTER I

Historical Introduction

OLIVER and SCHÄFER (1895) showed that intravenous administration of adrenal extract had a pronounced effect on cardiac activity, blood pressure, respiration, etc. Attempts to isolate the active principle of the adrenal medulla were successfully made by TAKAMINE (1901), who called the substance "adrenaline".

Further investigations were conducted by ELLIOTT (1904), who propounded the theory that adrenaline constituted the transmitter substance in sympathetic nerve endings. BARGER and DALE (1910) found, in their investigations, that the effect of stimulation of the sympathetic nerves was not fully consistent with the action of adrenaline, but in some cases was better reproduced by other homologous amino and ethylamino bases of catechols. LOEWI (1921) was able to demonstrate, in heart perfusate from frogs during stimulation of the sympathetic, the presence of a sympathicomimetic substance which he later (1936) identified as adrenaline. CANNON and URIDIL (1921) found that stimulation of the hepatic nerves liberated a sympathicomimetic substance which increased the blood pressure and caused acceleration of the heart rate but, in contrast to adrenaline, only moderate mydriasis. CANNON and BACQ (1931) named the substance liberated by stimulation of sympathetic nerves "sympathin". In view of the differences in physiological action between "sympathin" and adrenaline, CANNON and ROSENBLUETH (1933) elaborated the theory that adrenaline gave rise to two kinds of sympathin, E (excitatory) and I (inhibitory). BACQ (1934) pointed out that the inhibitory effect obtained by stimulation of sympathetic nerves showed similarities with that elicited by adrenaline, whereas the excitatory effect was comparable with that induced by noradrenaline. This theory was later abandoned by BACQ himself (1935), though STEHLE and ELLSWORTH (1937), MELVILLE (1937) and GREER *et al.* (1938) gave further support

to the idea. It was EULER (1946 a, b, c) who, in extracts from sympathetic nerves and visceral organs, first demonstrated a sympathicomimetic substance having the properties of noradrenaline. This fact, and the agreement in action between adrenergic nerve stimulation and noradrenaline, led EULER to conclude that noradrenaline constituted the sympathetic transmitter substance. His observations were verified by other authors. The occurrence of noradrenaline in adrenal extracts and in the urine was demonstrated by HOLTZ *et al.* (1947, 1948). Several authors confirmed these observations, and the chemical isolation of l-noradrenaline from fresh adrenal extracts was accomplished by BERGSTRÖM, EULER and HAMBERG (1949).

The biological effects of adrenaline and noradrenaline, although similar, present considerable differences. Noradrenaline generally has a weaker inhibitory effect, in comparison with equivalent amounts of adrenaline, on a large number of visceral organs (BARGER and DALE, 1910; EULER, 1946 c, 1948 a; and others).

The effect of noradrenaline on the isolated mammalian heart is, in general, similar to that of adrenaline. When injected into the human organism, the reflex bradycardia is secondary to the hypertensive action of noradrenaline (BARCROFT and KONZETT, 1949; KAPPERT *et al.*, 1950), whereas the effect of adrenaline generally is a primary increase of the pulse rate.

In contrast to adrenaline, which in physiological doses has a vasodilator effect associated with an increased cardiac output (EULER & LILJESTRAND, 1927), noradrenaline produces general vasoconstriction with very slight change in the cardiac output (GOLDENBERG *et al.*, 1948; EULER, review, 1951). Noradrenaline, in common with adrenaline, has a dilator effect on the coronary vessels (FOLKOW, FROST & UVNÄS, 1949).

The effect of noradrenaline on the oxygen consumption is very slight compared with that of adrenaline; this having been investigated both in man (GOLDENBERG *et al.*, 1948) and in small mammals, such as rats (THIBAULT, 1948), guinea pigs (LUNDHOLM, 1949) etc.

HOUSSAY and GERSCHMAN, (1947) obtained a pronounced glycogenolytic effect with both noradrenaline and adrenaline in experimental perfusion of isolated liver of toad.

SCHÜMANN (1949) found that the effect of noradrenaline on

the blood sugar was ten times weaker than that elicited with equivalent amounts of adrenaline.

EULER and LUFT (1952) found that insulin hypoglycemia in man gave rise to an increased excretion of adrenaline in the urine, whereas the excretion of noradrenaline was not increased. HÖKFELT (1951), in experiments on rats, observed that the adrenaline content in the adrenals declined following administration of insulin, and, with repeated doses of insulin, he was able (1953) to empty the adrenals of adrenaline in cats.

DUNÉR (1953), on elevating the blood sugar level in cats, observed a fall in the resting secretion of catechol amines in the adrenal venous blood. This effect was most pronounced on the adrenaline secretion, and could also be obtained by local injection of glucose in the hypothalamic region.

CHAPTER II

General Methods

Preparation and Purification of Extracts

The extraction method used here was EULER's modification (1948 b) of SHAW's specific adsorption method (1938), using trichloroacetic acid and subsequent adsorption of the catechol amines on aluminum hydroxide. In those cases where the biological determination was implemented by paper partition chromatography, further purification of the extract was done by evaporation and extraction of the residue with acetone.

A. Extraction Procedure

The specimens or tissues were mixed with 3 volumes of 8 per cent trichloroacetic acid for a few minutes in a Waring blender. After standing thirty minutes the extract was suction filtered. To facilitate filtration, 1 g "Celite" was added per 100 ml extract. To the clear filtrate was then added 20 per cent aluminum sulphate solution in sufficient quantity to make the final concentration of aluminum sulphate 0.2 per cent. The pH was thereafter adjusted to 7.5 by careful addition of 0.5 N NaOH while stirring. The aluminum hydroxide precipitate was collected on a suction filter, washed twice with 10 ml distilled water, and then dissolved in a few milliliters of 2 N H_2SO_4. Following adjustment of the pH to 3.5 with 0.5 N NaOH, the salts were precipitated by the addition of 4 volumes of a mixture of ethyl alcohol and acetone in equal parts. After storage in a refrigerator for between 4 and 15 hours the salts were filtered off, the filtrate concentrated *in vacuo* to a suitable volume, and the pH adjusted to 3.5. The finished extract was then stored in a deep-freeze until biological determination of the adrenaline and noradrenaline could be undertaken. In some of the invertebrate specimens, the salts were not removed until extracts had been stored for a few weeks in a deep-freeze. Standard solutions of adrenaline and noradrenaline which had been treated and stored

under similar conditions showed no demonstrable loss of activity, apart from that associated with the extraction procedure itself, in which the yield is approximately 75 per cent.

B. Further Purification

In those cases in which the biological assay indicated that sufficient amounts of catechol amines were present for chemical determination by paper partition chromatography, or in which paper partition chromatography with subsequent biological assay was desirable (*vide* Paper Partition Chromatography, B), the extract was washed with 1 volume of ether and then concentrated *in vacuo* until almost dry. The catechols were then extracted with about 100 volumes of anhydrous acetone. Following filtration the filtrate was once more concentrated until almost dry, then stored in a deep-freeze until paper partition chromatography could be carried out, which was usually within four hours. To avoid oxidation of the catechol amines during concentration *in vacuo*, the latter procedures were carried out at pH 2 — 3.5 in the presence of nitrogen.

Biological Assay

Quantitative determination of adrenaline and noradrenaline in extracts from organs was carried out by the method of EULER (1949), which is based on the fact that noradrenaline has a stronger effect than adrenaline on the cat's blood pressure, whereas adrenaline usually has a twenty to forty times greater relaxing action on the fowl's rectal cæcum.

In the present investigation biological assays showed an approximate mean error of 10—25 per cent for pure adrenaline and noradrenaline; which figures roughly approximate to those reported by HÖKFELT (1951) and DUNÉR (1953).

A. Cat's Blood Pressure

The cats were narcotized with 35 mg Pentobarbital sodium per kg body weight, administered intraperitoneally in a suitable amount of Ringer's solution. In order to stabilize the blood pressure, 2 mg of atropine sulphate per kg body weight was given subcutaneously and the sensitivity to pressor drugs was increased

by administering 0.1 mg ergotamine per kg body weight intramuscularly (EULER and SCHMITERLÖW, 1944). The blood pressure was recorded from one of the carotid arteries and injections were given into a femoral vein. After each injection 1—2 ml Ringer's solution was injected through the cannula. Fresh solutions of adrenaline and noradrenaline at pH 3.5 were used as standards. The activity ratio of adrenaline to noradrenaline on the cat's blood pressure, usually 0.4—0.8:1, was checked at regular intervals and generally varied only very slightly during the course of a single experiment.

B. Fowl's Rectal Cæcum

Following decapitation of the fowl, 3—4 cm of the rectal cæcum was removed and placed in a bath with a capacity of 20 ml. The Tyrode's solution contained 0.8 per cent NaCl, 0.01 per cent KCl, 0.02 per cent $CaCl_2$, 0.02 per cent $MgCl_2 \cdot 6\,H_2O$, 0.1 per cent $NaHCO_3$, 0.005 per cent $NaH_2PO_4 \cdot H_2O$, and 0.1 per cent glucose. It was aerated with a mixture of 94 per cent oxygen and 6 per cent carbon dioxide. The temperature was kept constant at 39°C.

The technique employed here usually enabled a pronounced relaxation of the preparation to be obtained by administration of 0.1—0.4 µg noradrenaline, while adrenaline was about thirty times more active (20:1 — 40:1).

Computation of the contents of adrenaline and noradrenaline in an extract was carried out as follows:

a = µg l-noradrenaline equivalents per g tissue on cat's blood pressure.

A = µg l-noradrenaline equivalents per g tissue on fowl's rectal cæcum.

q = activity ratio of adrenaline to noradrenaline on cat's blood pressure (< 1).

Q = activity ratio of adrenaline to noradrenaline on fowl's rectal cæcum (> 1).

$$\text{Adrenaline µg per g tissue} = x = \frac{A\text{-}a}{Q\text{-}q}$$

$$\text{Noradrenaline µg per g tissue} = y = A - xQ$$

$$\text{Per cent of adrenaline} = \frac{x}{x+y} \cdot 100$$

Paper Partition Chromatography

A. *Descending run with n-butanol* saturated with N HCl according to the method reported by EULER and HAMBERG (1949). The descending time was 15—24 hours. All paper partition chromatography was carried out at temperatures between 23 and 26°C. The paper used was Grycksbo OB.

The standard solutions consisted of adrenaline, noradrenaline, 3,4-dioxyphenylethylamine (dopamine), and 3,4-dioxyphenylalanine (dopa) dissolved in N HCl to a concentration of 5 mg per ml. Following chromatography the paper was dried at 60—70°C for 10—15 minutes, then sprayed with 0.1 per cent solution of ninhydrin in butanol. The paper was thereafter dried at a temperature of 90—100°C for 5—10 minutes or until the dopa standard gave a blue spot on the paper. A pale rose color was obtained with noradrenaline and dopamine after spraying with ninhydrin; but with the above procedure this color reaction did not occur until some hours had elapsed. On subsequent spraying of the paper with 0.44 per cent potassium ferricyanide at pH 7.7 (JAMES, 1948), and drying for twenty minutes at 60—70°C, adrenaline produced a rose-colored spot, but noradrenaline a purple, and dopamine and dopa a brownish-blue spot. If the temperature was high (90—100°C) during drying of the potassium ferricyanide, each of the catechols assumed more of a brownish-grey color.

On examination in ultraviolet light the characteristic fluorescence of adrenaline and noradrenaline could be observed even in the presence of such small amounts (1—2 μg) that no coloration by potassium ferricyanide was detectable in daylight. To obtain satisfactory fluorescence the paper was heated to 80—90°C for twenty minutes.

B. *Paper partition chromatography* was, in some cases, carried out by the technique just described, with subsequent division of the mid-section of the paper, elution in 5—10 ml of 0.05 N H_2SO_4, and biological assay for adrenaline and noradrenaline in the various sections of the paper (Fig. 1).

C. *Descending run with phenol* saturated with 0.1 N HCl. The descending time was 15—20 hours. Spraying was done as above (A) with ninhydrin and potassium ferricyanide. Paper partition

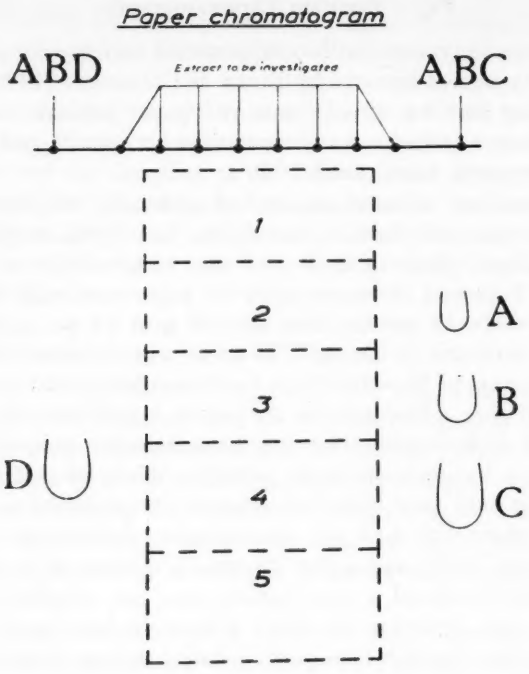


Fig. 1.

Fig. 1. Diagram showing paper chromatography according to B (see methods). Descending run with n-butanol saturated with N HCl. Time 15—25 hours. The standard solutions consisted of
(A) Noradrenaline, (B) Adrenaline, (C) Dopamine and (D) Dopa.
The side sections show the reaction of the standard solutions after spraying with a 0.1 per cent solution of ninhydrin in butanol (left), and 0.44 per cent potassium ferricyanide at pH 7.7 (right).
Each part of the middle section was eluted separately in a few milliliters of 0.05 N H_2SO_4 for biological determination of the content of catechol amines.

chromatography was carried out especially on those specimens which, on examination by the method described in section A, had been found to contain dopamine. With this technique the separation of dopamine and dopa was already complete after

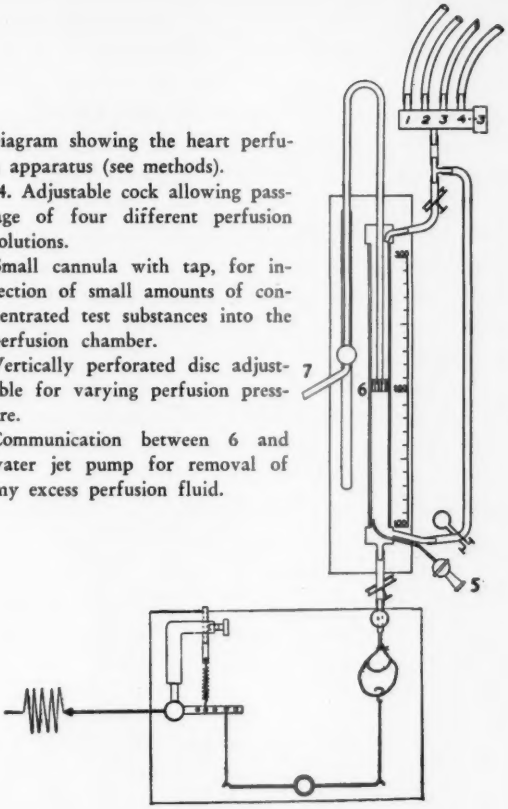


Fig. 2. Diagram showing the heart perfusion apparatus (see methods).
1—4. Adjustable cock allowing passage of four different perfusion solutions.
5. Small cannula with tap, for injection of small amounts of concentrated test substances into the perfusion chamber.
6. Vertically perforated disc adjustable for varying perfusion pressure.
7. Communication between 6 and water jet pump for removal of any excess perfusion fluid.

10—15 hours, so that even minute amounts of those substances could be differentiated and identified. The amount of dopamine in an extract was estimated approximately by comparing the color of the dopamine spot after spraying, with those of standard solutions of dopamine run on the same paper.

To avoid decomposition of the catechol amines, when phenol was the solvent, all paper partition chromatography was carried out in the presence of an atmosphere of nitrogen.

Heart Perfusion

With the heart *in situ* a cannula was inserted in one of the entering veins, the others then being ligated. The heart was thereafter dissected out and placed in the perfusion apparatus (Fig. 2). The perfusion pressure was kept constant, generally at about 150 mm water, the level depending on the volume of the heart. An excess of fluid was continuously supplied to the apparatus, so that the heart received more fluid when its activity increased. The level of the fluid was kept constant by means of a water jet pump. Heart movements were recorded on a smoked drum via a system of levers in which the heart contracted against the resistance of an adjustable coil spring connected to the recording pen. The perfusion fluids, mainly based on sea water, are described later. Due to the pH of the perfusion solution, which was often over 8, the adrenaline in particular was rapidly destroyed following dilution, so that all diluting was done immediately before the tests. Breakdown of catechol amines could also be avoided by injecting them in concentrated form directly into the perfusion chamber. This gave an instantaneous dilution in a small volume of fluid which passed through the heart before any appreciable breakdown could occur.

Substances Used

Adrenaline hydrochloride. Synthetic adrenaline base (Rhone—Poulenc, Paris) was dissolved in N/10 HCl, diluted with distilled water and the pH adjusted to about 4.5.
l-Noradrenaline hydrochloride. Synthetic l-noradrenaline bitartrate (Winthrop—Stearns Inc., New York) was dissolved, diluted, and the pH adjusted as with adrenaline.
Acetylcholine chloride (Hoffman—La Roche, Basel).
Antihistamine; Lergigan, A. B. Recip, Stockholm was used.
Atropine sulphate — commercial samples.
Cocaine hydrochloride — commercial samples.
Dihydroergotamine. (Sandoz, Basel).
Dopa (3,4-dioxyphenylalanin). (Hoffman—La Roche, Basel).

Dopamine (3,4-dioxyphenylethylamine)=Hydroxytyramine hydrochloride (Sterling—Winthrop Research Institute, New York).
Ergotamine tartrate (Gynergen, Sandoz, Basel).
Histamine dihydrochloride (Hoffman—La Roche, Basel).
5-Hydroxytryptamine (Serotonin Creatinine Sulfate, Abbot Laboratories, North Chicago).
"Celite" *Hyflo Super Cel* (Johns—Manville, U. S. A.).
Pentobarbital sodium (Nembutal, Abbot, London).
Physostigmin. salicylic. cryst. (E. Merck, Darmstadt).
Tyramine hydrochloride (Hoffman—La Roche, Basel).

CHAPTER III

Catechol Amines in Invertebrates (other than Insects)

Earlier Investigations

LEYDIG (1857) observed in the ganglia of the leech *Pontobdella verucosa* and other closely related invertebrates, some curious cells, containing yellow granules, that he believed to be analogous with the adrenal cells of higher animals. RETZIUS (1891) and others described the cells in greater detail, though without referring to any chromaffin characteristics they may have had.

The occurrence of chromaffin cells in annelids was demonstrated by POLL & SOMMER (1903) and POLL (1908), who found that some nerve cells in the ganglia of leeches — the medicinal leech *(Hirudo medicinalis)*, terrestrial leech *(Aulastomum gulo)* and turtle leech *(Placobdella catenigra)* — showed a chromaffin reaction similar to that obtained with cells of the adrenal medulla. BIEDL (1910) tested extracts from the ventral ganglionic chains of the leech and earthworm and concluded that they contained adrenaline. GASKELL (1914, 1919), who investigated numerous specimens of annelids, found that chromaffin cells occurred only in those possessing contractile vascular systems. After dissecting out central ganglia from the medicinal leech and performing extraction he showed — by biological tests on pregnant cat uteri treated with histamine — that the extract contained a substance whose characteristics were apparently those of adrenaline. According to him, "The sympathetic nervous system and the adjuvant adrenaline-secreting system are found in their earliest form in the annelid kingdom, and consist of cells situated in the central nervous system which are both secretory and nervous in function. The contractile vascular system of annelids is mainly branchial in function. The vertebrate heart has been derived from it by the growing around of the lateral body folds to form a new ventral surface."

Some of GASKELL's observations were verified by VIALLI (1934),

WENSE (1938 a), LANCASTER (1939), and PEREZ (1942). EULER (1948/49) observed in extracts from the earthworm sympathicomimetic activity that differed from that of pure adrenaline and was more consistent with the behavior of a mixture of adrenaline and noradrenaline.

Investigations on other invertebrates are sparse. BAYER & WENSE (1936) claimed that extracts from *Paramecium* contained a sympathicomimetic substance which, in biological tests on atropinized frog heart and rabbit gut, before and after cocaine, appeared to be adrenaline. The extracted substance also behaved like adrenaline with regard to inactivation by oxygen, staining green with ferrichloride, and fluorescing in ultraviolet light. ROAF & NIERENSTEIN (1907) extracted a hypertensive substance from the hypobranchial gland of the purple *(Purpura lapillus)*, and ROAF (1911) found that chromaffin cells were present in the purple's gland, while extracts from that animal gave a green color with ferrichloride. LISON (1932) declared, however, that the cells described by ROAF were pseudochromaffin and not really adrenaline-secreting cells.

EULER (1952) found no detectable catechol amines in molluscs, crustaceans and echinoderms, with the exception of the posterior salivary gland of the common European octopus *(Octopus vulgaris)*, which contained comparatively large amounts of noradrenaline. This gland, as shown by ERSPAMER and BORETTI (1951), contains octopamine, which is readily transformed into noradrenaline by ultraviolet irradiation.

The occurrence of amine oxidase in invertebrates has been investigated especially by BLASCHKO and his associates (BLASCHKO, review, 1952). Amine oxidase has been demonstrated in annelids (BLASCHKO & HIMMS, 1953). Two echinoderms that were investigated — the starfish *Asterias rubens* and the sea urchin *Echinus esculentus* — also contained amine oxidase. The enzyme occurred in the limpet *Patella vulgaris*, but not in the snail *Helix aspersa*, (BLASCHKO, RICHTER & SCHLOSSMANN, 1937). A detailed investigation by BLASCHKO & HAWKINS (1952), into the occurrence and distribution of amine oxidase in the cuttlefish *(Sepia officinalis)* and octopus *(Octopus vulgaris)*, showed that those animals "contained in their hepatopancreas the richest source of amine oxidase yet described".

Reviews on the occurrence and action of hormones in invertebrates have been published by a number of investigators, including KOLLER (1938), WENSE (1938 b), HANSTRÖM (1939), BACQ (1947), WIGGLESWORTH (1953) and SCHARRER (1953).

Material

The following species of invertebrates were investigated for their contents of catechol amines:

A.	*Protozoa*	*Noctiluca*	*(Noctiluca miliaris)*
B.	*Coelenterata*	1. Dead man's fingers	*(Alcyonium digitatum)*
		2. Sea anemone	*(Metridium dianthus)*
		3. Jellyfish	*(Rhizostoma pulmo)*
C.	*Echinoderma*	1. Sea cucumber	*(Mesothuria intestinalis)*
		2. Sea urchin	*(Echinus esculentus)*
		3. Brittle star	*(Ophiura albida)*
D.	*Annelida*	1. Lugworm	*(Arenicola marina)*
		2. Earthworm	*(Lumbricus terrestris)*
E.	*Arthropoda*	1. Water flea	*(Daphnia pulex)*
	(Crustacea)	2. Shrimp	*(Crangon crangon)*
F.	*Mollusca*	1. Common whelk	*(Buccinum undatum)*
		2. Common mussel	*(Mytilus edulis)*
		3. Edible snail	*(Helix pomatia)*
G.	*Tunicata*	Tube sea squirt	*(Ciona intestinalis)*

Freshly caught organisms were freed from contaminating matter and shells, and the intestinal contents removed. The bulk of the excess water was pressed out prior to weighing and mixing with 3 volumes of 8 per cent trichloroacetic acid. Following adsorption and removal of the aluminum salts the extracts were then stored in a deep-freeze until tested biologically according to the methods previously described (Chapter II). Extracts from water fleas *(Daphnia pulex)*, edible snails *(Helix pomatia)* and

earthworms *(Lumbricus terrestris)* were prepared and biologically tested directly after collection. With the exception of earthworms — in which the ventral ganglionic chain was dissected out under the microscope and then extracted — all extractions were carried out on whole animals.

For further purification and paper partition chromatography the following were selected, due to the limited amount of material available: *Noctiluca miliaris, Metridium dianthus, Mesothuria intestinalis, Crangon crangon and Mytilus edulis.*

Perfusion experiments on the isolated squid heart are described in Chapter V.

Results

1. *Earthworms.* Extracts from ganglionic chains removed from earthworms produced a rise of blood pressure and a relaxing

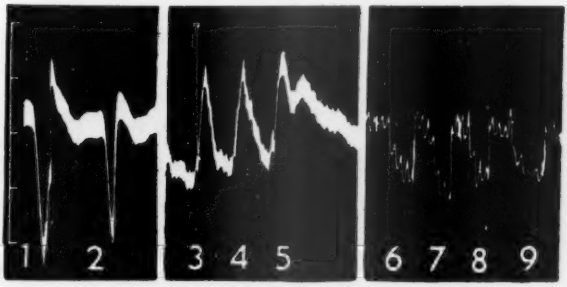


Fig. 3.

Fig. 3. Effect of earthworm extract on cat's blood pressure (1—5) and fowl's rectal cæcum (6—9). Ordinate 80—160 mm Hg. q = 0.2; Q = 20. All figures refer to wet weight.

1. Whole body extract corresponding to 5 g tissue.
2. Whole body extract corresponding to 2.5 g tissue.
3. 0.1 µg noradrenaline.
4. 0.5 µg adrenaline.
5. Extract from ventral ganglionic chain corresponding to 0.2 g tissue.
6. 0.01 µg adrenaline.
7. 0.25 µg noradrenaline.
8. 0.2 µg noradrenaline.
9. Extract from ventral ganglionic chain corresponding to 0.07 g tissue.

Table 1

Biological Effect on Cat's Blood Pressure and Fowl's Rectal Cæcum of Whole Body Extract from Invertebrates. Trichloroacetic Acid Extraction. (*Cf.* Figs. 3—5.)

Species	Number of Animals	Total Weight of Tissue in G	Cat's Blood Pressure				Adrenaline Equivalents in mμg/g on Fowl's Rectal Cæcum
			Amount Given G Tissue	Initial Depressor Effect	Secondary Pressor Effect	Adrenaline Equivalents in mμg/g	
Noctiluca	—	500	30	+	0		0.4
Dead man's fingers	—	650	60	+	+		1.5
Sea anemone	25	380	50	+	++	2	2.0
Jellyfish	2	3000	100	+	+		0.3
Sea cucumber	75	500	10	+++	0		1.0
Sea urchin	5	170	50	+	++	4	7.5
Brittle star	150	300	12	0	0		1.2
Lugworm	50	120	12	+++	++		32
Earthworm	18	96	5	+++	++		45
Water flea	—	100	20	++	+		5
Shrimp	—	2000	50	+	+		1.7
Common whelk	22	400	40	+	++	6	0.7
Common mussel	42	470	50	+	+		4
Edible snail	35	300	15	0	0		4
Tube sea squirt	—	860	25	0	0		0.8

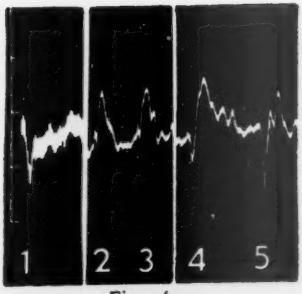


Fig. 4.

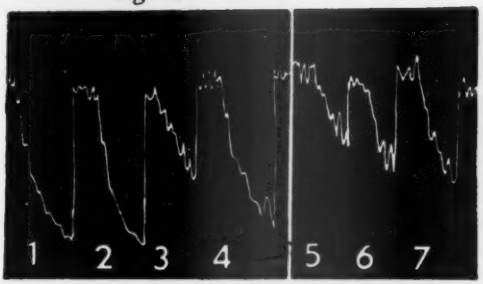


Fig. 5.

Fig. 4. Cat's blood pressure. Ordinate 80—160 mm Hg. q = 0.5.
1. Extract corresponding to 20 g *Noctiluca* (whole body extract) (*Cf.* Table 1).
2. 0.1 µg noradrenaline.
3. 0.2 µg adrenaline.
4. Whole body extract from common whelk corresponding to 40 g tissue.
5. Whole body extract from sea anemone corresponding to 40 g tissue.

Fig. 5. Fowl's rectal cæcum. Q = 30 (1—4) and 20 (5—7).
1. 0.3 µg noradrenaline.
2. 0.01 µg adrenaline.
3. Extract corresponding to 2 g tissue of *Noctiluca.*
4. Extract corresponding to 4 g tissue of *Noctiluca.*
5. Extract corresponding to 8 g tissue of common whelk.
6. 0.25 µg noradrenaline.
7. Extract corresponding to 12 g tissue of common whelk.

effect on fowl's rectal cæcum corresponding to 1.4 µg adrenaline and 0.32 µg noradrenaline per g tissue wet weight. Extracts from whole earthworms, in common with extracts prepared in the same way from some of the other invertebrates, produced an irregular, frequently hypotensive, effect (Fig. 3).

2. *Other Invertebrates.* A satisfactory biological analysis for adrenaline and noradrenaline was not always possible on account of the threshold readings obtained in many cases as well as the atypical effects produced by some extracts.

As a rule, amounts of extracts corresponding to 5—100 g tissue wet weight were either inactive or produced a fall in blood pressure, which was unchanged after administration of atropine, antihistaminics or cocaine. In some cases the initial fall in blood pressure was followed by an apparently specific rise (Figs. 4 and 5, Table 1).

Most extracts had a relaxing effect on the fowl's rectal cæcum corresponding to 0.2—30 mμg adrenaline per g tissue wet weight. Extracts from *Noctiluca miliaris, Alcyonium digitatum, Mesoturia intestinalis, Mytilus edulis* and *Ciona intestinalis* had, in addition, a desensitizing effect against standard solutions of adrenaline and noradrenaline. This effect disappeared if an interval of five minutes was allowed between the addition of the extract and the standard solutions of adrenaline and noradrenaline.

Paper partition chromatography (Type A) was carried out on *Noctiluca miliaris, Metridium dianthus, Mesoturia intestinalis, Crangon crangon* and *Mytilus edulis.* After a descending run of 20 hours and spraying with ninhydrin, no spots were detectable; but with potassium ferricyanide, extracts from *Noctiluca miliaris, Metridium dianthus* and *Mytilus edulis* produced distinct rose-colored spots having low R_f values. Since the solvent front passed off the paper in the descending time used, it was difficult to calculate the R_f value, which, however, was not consistent with standard solutions of adrenaline, noradrenaline, dopamine, dopa, tyrosine or 5-hydroxytryptamine, and was approximately half the R_f value obtained with noradrenaline. (Fig. A.) The R_f value when using phenol saturated with 0.1 N HCl was 0.02—0.03. This spot, on examination in ultraviolet light, showed a blue color with a faint bluish white fluorescence. Extracts of section I (Fig. 1) in which the spot was situated, gave the greatest relaxing activity on fowl's rectal cæcum. The substance giving this unidentified spot has also been detected in several other animals, as will be described later. It is likely to be a catechol amine as the adsorption method is thought to be specific for this group of

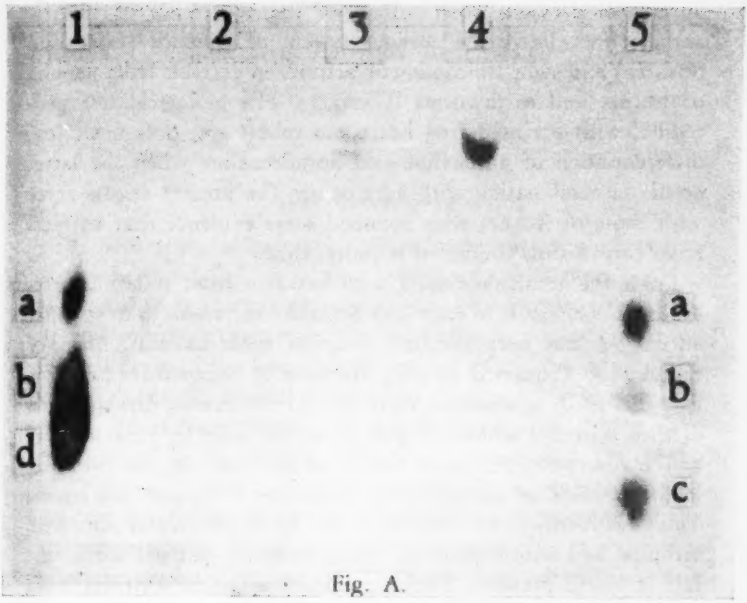


Fig. A.

Fig. A. Paper chromatography of purified extracts from some invertebrates. Solvent n-butanol saturated with N HCl. Descending time 17 hours. Sprayed with 0.1 per cent ninhydrin and 0.44 per cent potassium ferricyanide.

1 and 5. Standard solutions of noradrenaline (a), adrenaline (b), dopamine (c) and dopa (d).

2. Purified shrimp extract corresponding to 200 g tissue.

3. Purified common mussel extract corresponding to 200 g tissue.

4. Purified sea anemone extract corresponding to 200 g tissue.

substances (EULER, 1952). Certainly it is not adrenaline, noradrenaline or dopamine. For convenience in reference it is thus proposed to name this unidentified substance "catechol-4", although it is admitted that its catechol nature is not established.

Discussion

The results described in this chapter seem to warrant the conclusion that the sympathicomimetic effects produced by extracts from ganglionic chains of earthworms are attributable to a mixture of adrenaline and noradrenaline. This is in good accord-

ance with previous observations on the occurrence of specific chromaffin cells in the nervous system of annelids (POLL and SOMMER) and sympathicomimetic activity in extracts from ganglia of leeches and earthworms (GASKELL). His biological test procedure, with perfused frog heart and rabbit gut, does not allow differentiation of adrenaline and noradrenaline when the latter occurs in combination with adrenaline. The present results agree with those of EULER, who obtained some evidence that extracts from earthworms contained noradrenaline.

From the results obtained with extracts from other invertebrates, it is difficult to draw any definite conclusions as to whether adrenaline and noradrenaline occur in those animals. Most of the extracts contained varying amounts of hypotensive activity, and this made evaluation difficult. At all events, the amounts of these catechol amines, if present at all, must be very low.

The adsorption procedure employed here rules out the presence, in the extracts, of acetylcholine, histamine or similar substances. This was verified by treatment of the experimental cat with atropine and antihistaminics, which measures did not affect the fall in blood pressure. The hypotensive effect of extracts from lower animals, previously observed by EULER, was confirmed in these investigations; but the nature of this substance is unknown.

The extracts, as will be seen from Table 1, contained varying amounts of a substance whose effect on fowl's rectal cæcum accorded with that produced by catechol amines. The few experiments with paper partition chromatography suggest that extracts from invertebrates contain a new substance with color reactions resembling those of the catechol amine which has been named catechol-4.

CHAPTER IV

Catechol Amines in Insects

Earlier Investigations

The occurrence of catechol amines in the mealworm has previously been studied in a few investigations. WENSE (1938 a) reported that extracts from mealworms contained a sympathicomimetic substance behaving like adrenaline in experiments on perfused heart of the frog and on isolated rabbit gut. WENSE also isolated chemically a small amount of a substance which, as regards melting point, inactivation by acetaldehyde, and sublimation, was identical with synthetic adrenaline. GREGERMAN and WALD (1952) tested WENSE's observations and, by colorimetric determination and paper chromatography following adsorption of the catechol amines on aluminum hydroxide, were able to show that the product isolated by him could not have been adrenaline. After paper chromatography and spraying with potassium ferricyanide they obtained, however, two spots that were possibly attributable to two ortho-diphenols which had previously been demonstrated by other methods in extracts from mealworms, namely, 3,4-dihydroxyphenylacetic acid, isolated by SCHMALFUSS, HEIDER & WINKELMANN (1933), and 3,4-dihydroxyphenyllactic acid, isolated by PRYOR, RUSSEL and TODD (1947). CAMERON (1953) stated in a preliminary report that with a technique similar to that employed by GREGERMAN and WALD he could obtain a third spot, by paper chromatography, provided he carried out extraction, adsorption and purification of the extracts within a few hours. This third spot contained a substance acting on chromatophores of crustaceans and comparable with extracts from the corpus cardiacum. The latter organ, he also found, gave a positive chromaffin reaction. His results led him to conclude that insects do not secrete adrenaline but another orthodiphenol having the function of adrenaline in the insect body. The chemical nature of this substance has not been elucidated. CAMERON's investigations have also been briefly communicated by WIGGLESWORTH (1954).

The occurrence and significance of orthodihydroxyphenols in insects have been investigated and discussed by PRYOR (1940 a, b), PRYOR, RUSSEL and TODD (1946), and HACKMAN, PRYOR and TODD (1948). These authors found that the above 3,4-dihydroxyphenols commonly occurred in insects and suggested that they may be essential to the formation of the exoskeleton. HACKMAN (1953), however, has reported experimental results which suggest that normal exoskeleton formation may occur in insects in the absence of orthodihydroxyphenols.

Material

The occurrence of catechol amines was investigated in the following insects:

A.	*Dermaptera*	Earwig, imago	*(Forficula sp.)*
B.	*Lepidoptera*	Larvae of small tortoiseshell butterfly	*(Vanessa urticae)*
C.	*Coleoptera*	Meal beetle, mealworm	*(Tenebrio molitor)*[1]
D.	*Diptera*	Common housefly and larvae	*(Musca domestica)*
E.	*Hymenoptera*	Honey bee, larvae, pupae, workers and drones	*(Apis mellifica)*

The insects were collected, sorted and weighed. Following grinding in a mortar, together with 3 volumes of 8 per cent trichloroacetic acid, the catechol amines were adsorbed on aluminum hydroxide at pH 7.5 as previously described. In the case of bigger preparations, such as worker bees and mealworms, the mixing was done in a Waring blender. Loss of catechol amines, which occurred on a few occasions during preparation, could be avoided by adding 0.5 ml analytically pure octanol per 100 ml of the trichloroacetic acid.

Some of the mealworms were divided prior to extraction. The two halves of the bodies were placed, immediately after division,

[1] I am indebted to Agronom R. Mathlein Stockholm who supplied me with some of these insects.

in separate tared vessels containing trichloroacetic acid with octanol added, after which preparation was carried out as before.

When preparation was complete — usually within 8—12 hours — the extracts were stored in a deep-freeze. Following biological determination and further purification, the extracts from all insects were submitted to paper partition chromatography, with butanol-HCl as the solvent (*vide* Paper Partition Chromatography A). Paper chromatography with phenol-HCl and butanol-HCl was also carried out with extracts from honey bee larvae and worker bees as well as from mealworms (*vide* Paper Partition Chromatography B and C). All distillations *in vacuo* with the exception of the last one were carried out in the presence of small amounts of octanol.

Results

In all extracts from insects sympathicomimetic effects were observed on cat's blood pressure and fowl's rectal cæcum which closely accorded with those of mixtures of adrenaline and noradrenaline (Figs. 6—10). These substances too, as well as dopamine, were demonstrable by paper chromatography. The extracts, with the exception of those from housefly larvae, also contained small amounts of a substance which, with phenol-HCl, *(Coleoptera and Hymenoptera)* had an R_f value of 0.02—0.03. On spraying with potassium ferricyanide this spot showed a pale rose color and a bluish-white fluorescence in ultraviolet light. The R_f value, color reaction with potassium ferricyanide and fluorescence in ultraviolet light, as well as the feeble sympathicomimetic effect on fowl's rectal cæcum were the same as those of the unidentified catechol-4 described in chapter III. In mealworms divided into anterior and posterior portions, catechol-4 occurred only in the anterior part.

A. *Dermaptera* Earwig *(Forficula sp.)*

The imagos contained 1.2 µg noradrenaline and 0.010 µg adrenaline per g body weight (Fig. 6, Table 2). Paper chromatography of a purified extract corresponding to 5 g of tissue confirmed the presence of noradrenaline, and in addition an amount of dopamine equivalent to < 1 µg per g body weight was present.

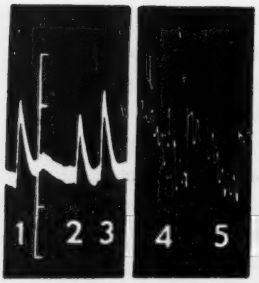


Fig. 6.

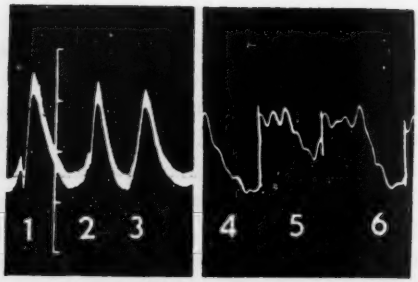


Fig. 7.

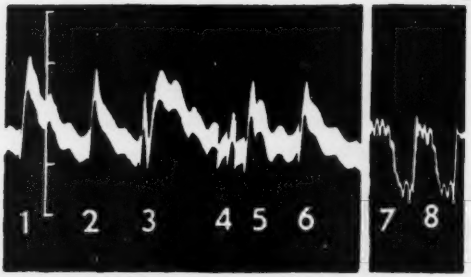


Fig. 8.

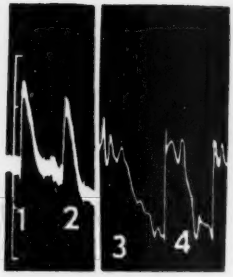


Fig. 9.

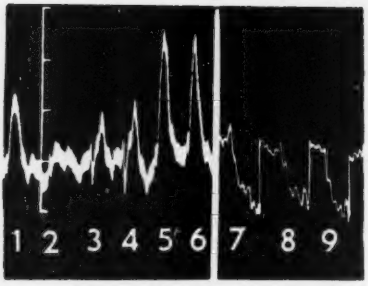


Fig. 10.

Fig. 10. Mealworm. (*Cf.* Fig. 11, Table 3.) q = 0.65; Q = 22. Ordinate 80—160 mm Hg.

1. 0.05 µg noradrenaline.
2. Extract from the anterior portion corresponding to 0.04 g tissue.
3. Extract from the anterior portion corresponding to 0.2 g tissue.
4. Extract from the anterior portion corresponding to 0.4 g tissue.
5. Extract from the posterior portion corresponding to 0.1 g tissue.
6. 0.1 µg noradrenaline.
7. Extract from the posterior portion corrresponding to 0.1 g tissue.
8. Extract from the anterior portion corresponding to 0.065 g tissue.
9. 0.3 µg noradrenaline.

Figs. 6—9. Biological assay on cat's blood pressure and fowl's rectal cæcum of whole body extracts from some insects (*Cf.* Table 2). Blood pressure ordinate 80—160 mm Hg.

Fig 6. Earwig. q = 0.45; Q = 20.
1. Extract corresponding to 0.2 g tissue.
2. 0.2 µg noradrenaline.
3. 0.3 µg noradrenaline
4. Extract corresponding to 0.1 g tissue.
5. 0.15 µg noradrenaline.

Fig. 7. Honey bee. q = 0.9; Q = 30.
1. Extract corresponding to 0.3 g tissue.
2. 0.2 µg noradrenaline.
3. 0.2 µg adrenaline.
4. 0.6 µg noradrenaline.
5. Extract corresponding to 0.1 g tissue.
6. Extract corresponding to 0.2 g tissue.

Fig. 8. Small tortoiseshell butterfly larva. q = 0.3; Q = 33.
1. Extract corresponding to 1 g tissue.
2. 0.15 µg noradrenaline.
3. 0.5 µg adrenaline.
4. 15 µg dopamine.
5. 30 µg dopamine.
6. Extract corresponding to 0.8 g tissue.
7. 0.15 µg noradrenaline.
8. Extract corresponding to 0.4 g tissue.

Fig. 9. Common housefly. q = 0.45; Q = 25.
1. Extract corresponding to 0.2 g tissue.
2. 0.3 µg noradrenaline.
3. Extract corresponding to 0.016 g tissue.
4. 0.15 µg noradrenaline.

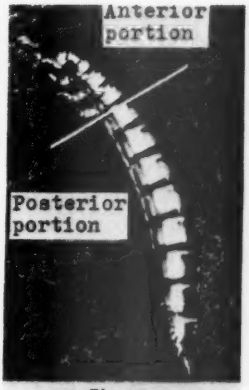


Fig. 11.

Fig. 11. Showing mode of division of the mealworm. *(Cf.* Table 3, Fig. 10).

Table 2

Adrenaline and Noradrenaline Content in Insects. Whole Body Extracts with Trichloroacetic Acid. Biological Assay on Cat's Blood Pressure and Fowl's Rectal Cæcum. (*Cf.* Figs. 6—9.)

Species		Number of Insects	Total Body Weight in G	Noradrenaline µg/g	Adrenaline µg/g	Adrenaline as Percentage of Adrenaline and Noradrenaline	Dopamine µg/g (Approximate)
Earwig	imago	65	5.5	1.2	0.010	1	<1
Small tortoiseshell butterfly	larva	—	17	0.19	<0.010	—	10—15
Meal beetle	(imago)	150	6	1.1	0.15	12	2—4
Mealworm	(larva)	250	25	1.3	0.021	2	10—15
»	»	500	20	2.2	0.061	3	10—15
Common housefly	imago	375	7.5	1.9	0.30	14	10—15
» »	larva	—	8	0.10	<0.010	—	4—8
Honey bee (worker)	imago	200	25.1	0.75	0.050	6	5—10
» » »	mature pupa	30	4.1	0.22	0.026	11	2—4
» » »	immature pupa	100	13.9	0.045	0.049	52	?
» » »	larva	155	17.6	0.30	<0.010	—	2—4
» » (drone)	imago	17	5.1	0.33	0.010	3	5—10
» » »	immature pupa	100	36.3	0.10	0.013	12	?
» » »	larva	103	36.8	0.76	0.075	9	5—10

B. *Lepidoptera* Small tortoiseshell butterfly *(Vanessa urticae)*

The larvae contained 0.19 μg noradrenaline and < 0.010 μg adrenaline per g body weight (Fig. 8, Table 2). Here, too, noradrenaline and dopamine were demonstrated chromatographically. The amount of dopamine was equivalent to 10—15 μg per g body weight.

C. *Coleoptera* 1. Meal beetle *(Tenebrio molitor)*

The insects contained 1.1 μg noradrenaline and 0.15 μg adrenaline per g body weight. Paper chromatography of a pure extract corresponding to 5 g of tissue showed the presence of noradrenaline and dopamine. The spot due to dopamine was equivalent to 2—4 μg per g body weight. (Table 2).

2. Mealworm *(Tenebrio molitor)*

The larvae contained 1.3—2.2 μg noradrenaline and 0.021—0.061 μg adrenaline per g body weight (Table 2). Extracts representing 5—15 g tissue, when studied by paper partition chromatography with butanol-HCl and phenol-HCl, were found to contain noradrenaline and dopamine. The dopamine spot corresponded to 10—15 μg per g tissue (Fig. B). When using more highly concentrated extracts, adrenaline was also demonstrable chromatographically.

The anterior portion (head and first four segments) contained only small amounts of noradrenaline — namely, 0.011—0.050 μg per g tissue — while the adrenaline content was comparatively high — 0.15—0.18 μg per g tissue. In extracts from the posterior portion (last eight segments) the reverse was true, with a high noradrenaline content of 0.93—0.94 μg and a low adrenaline content of 0.093—0.094 μg per g tissue. (Figs. 10 and 11, Table 3). The amount of dopamine was estimated at 1—4 μg per g tissue in the anterior and 10—15 μg per g in the posterior portion. Paper chromatography of extracts from the anterior portion showed a spot consistent with the presence of noradrenaline in a concentration of 0.1—0.2 μg per g tissue (Fig. C).

Extracts from mealworms were also submitted to paper chromatography in combination with biological testing of horizontal sections of paper (according to B). The section that corresponded to the site of noradrenaline contained a factor which in every respect was comparable to a standard solution of noradrenaline.

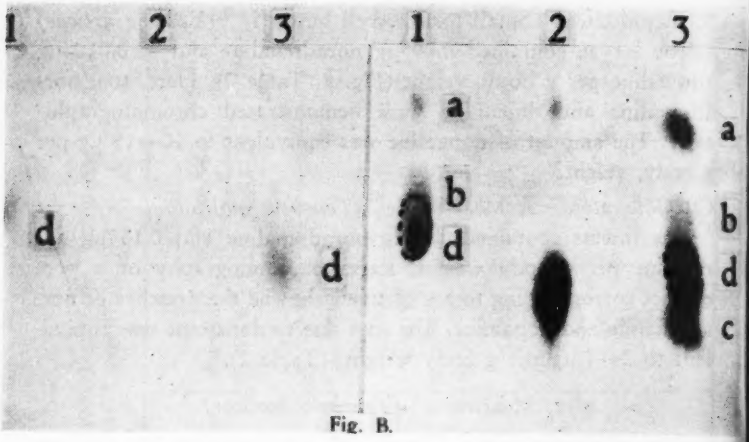


Fig. B. Paper chromatography of purified extract from mealworm. Solvent n-butanol N HCl. Descending time 20 hours.
The figure shows two photographs of the same paper chromatogram. (Left), After spraying with 0.1 per cent ninhydrin; (right), after spraying with 0.44 per cent potassium ferricyanide.
1 and 3. Standard solutions of noradrenaline (a), adrenaline (b), dopamine (c) and dopa (d).
2. Extract from mealworm corresponding to 25 g tissue.

Table 3

Adrenaline and Noradrenaline Content in the Anterior and Posterior Portion of the Mealworm. Trichloroacetic Acid Extraction. Biological Assay on Cat's Blood Pressure and Fowl's Rectal Cæcum. (*Cf.* Figs. 10—11.)

Tissue	Number of Larvae	Total Weight of Tissue in G.	Noradrenaline µg/g	Adrenaline µg/g	Adrenaline as Percentage of Adrenaline and Noradrenaline	Dopamine µg/g (Approximate)
Anterior portion	350	12	0.050	0.15	75	1—4
Anterior portion	660	25	0.011	0.18	95	1—4
Posterior portion	350	29	0.94	0.093	9	10—15
Posterior portion	660	50	0.93	0.094	9	10—15

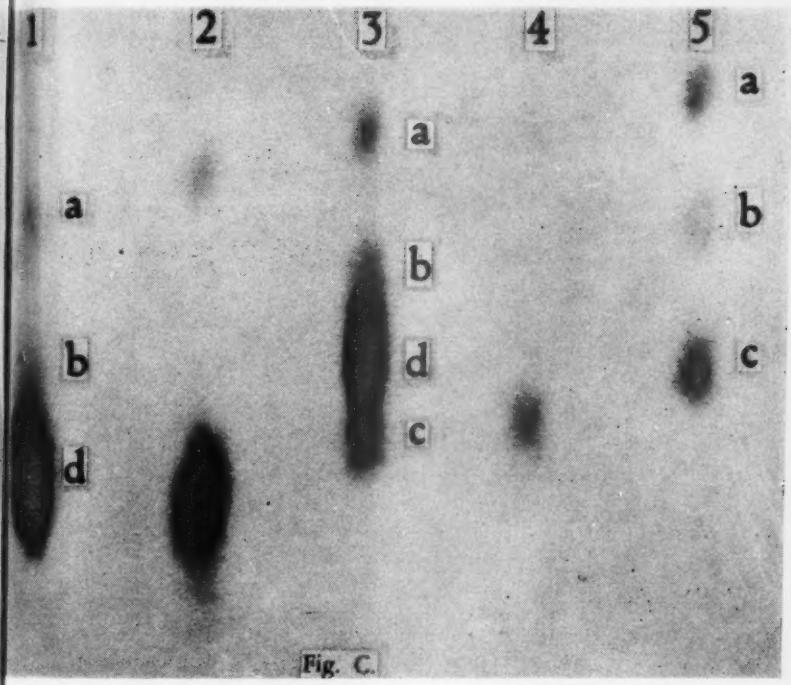


Fig. C.

Fig. C. Paper chromatography of extracts from the anterior and posterior portions of the mealworm.
Solvent n-butanol saturated with N HCl. Descending time 22 hours. Photograph taken after spraying with 0.44 per cent potassium ferricyanide.
The solvent front was oblique.

1, 3 and 5. Standard solutions of noradrenaline (a), adrenaline (b), dopamine (c), and dopa (d).

2. Purified extract from the posterior portion of the mealworm corresponding to 20 g tissue.

4. Purified extract from the anterior portion of the mealworm corresponding to 15 g tissue.

D. *Diptera* Common housefly *(Musca domestica)*

The imagos contained 1.9 µg noradrenaline and 0.30 µg adrenaline per g body weight. The larvae, which varied in length from 3 to 12 mm, contained only a little hypertensive activity, preceded by a fall in blood pressure. The approximate contents of nor-

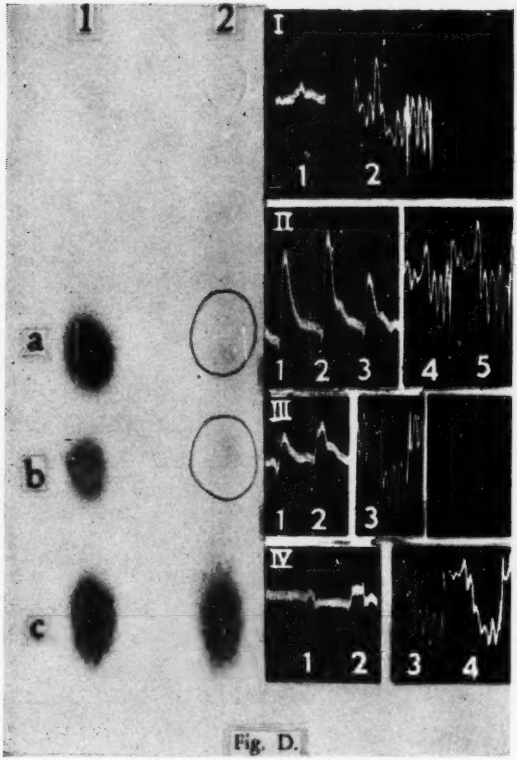


Fig. D.

Fig. D. Paper chromatography combined with biological testing of horizontal sections (according to B). Solvent n-butanol-HCl. Descending time 22 hours. Sprayed with potassium ferricyanide. Material: Extract corresponding to 35 g worker bees.

To the left:

1. Standard solutions of noradrenaline (a), adrenaline (b) and dopamine (c).
2. Extract corresponding to 15 g worker bees. (The ringed areas indicate spots observed in ultraviolet light.)

To the right:

Biological assay on cat's blood pressure and fowl's rectal cæcum of eluates from sections I, II, III, and IV. Each section eluted with 10 ml 0.05 N H_2SO_4.

Section I. 1. 2 ml eluate on cat's blood pressure.
2. 2 ml eluate on fowl's rectal cæcum.

Section II. 1. 1 ml eluate on cat's blood pressure.
2. 0.2 µg noradrenaline.

3. 0.1 μg noradrenaline.
4. 0.1 μg noradrenaline on fowl's rectal cæcum.
5. 1 ml eluate on fowl's rectal cæcum.

Section III. 1. 2 ml eluate on cat's blood pressure.
2. 0.15 μg adrenaline.
3. 0.5 ml eluate on fowl's rectal cæcum.

Section IV. 1. 0.5 ml eluate on cat's blood pressure.
2. 15 μg dopamine.
3. 0.5 ml eluate on fowl's rectal cæcum.
4. 4 μg dopamine.

adrenaline and adrenaline were respectively 0.10 μg and < 0.010 μg per g body weight. (Fig. 9, Table 2.)

Paper chromatography with a purified extract made from 5 g of imagos showed noradrenaline and an amount of dopamine corresponding to 10—15 μg per g body weight. Extract from 6 g of larvae, studied in the same way, showed 4—8 μg dopamine per g body weight as well as noradrenaline.

E. *Hymenoptera* Honey bee *(Apis mellifica)*

1. Workers. — Five-day-old larvae were found to contain 0.30 μg noradrenaline and < 0.010 μg adrenaline per g body weight. Immature pupae had contents of 0.045 μg noradrenaline and 0.049 μg adrenaline per g body weight. Mature pupae, immediately before emergence of the imagos, contained 0.22 μg noradrenaline and 0.026 μg adrenaline per g body weight, whereas the adult workers contained 0.75 μg and 0.050 μg respectively. (Fig. 7, Table 2.)

2. Drones. — The larvae contained 0.76 μg, immature pupae 0.10 μg and imagos 0.33 μg noradrenaline per g body weight; the corresponding contents of adrenaline were 0.075 μg, 0.013 μg and 0.010 μg respectively.

In the worker bee noradrenaline and dopamine were demonstrated chromatographically, the content of dopamine being 5—10 μg per g body weight for the imagos and 2—4 μg for the larvae. Paper chromatography combined with biological testing of horizontal sections showed an accumulation of hypertensive activity in the sector coinciding with the site of noradrenaline. The extract was from 20 g of worker bees, containing about 15 μg noradrenaline. Following paper chromatography 1.5 μg noradrenaline, or 10 per cent, was recovered. (Fig. D.)

Discussion

The results reported in this chapter show that the three catechol amines, adrenaline, noradrenaline and dopamine, occur in appreciable quantities in various insects.

In view of the paucity of the data reported by WENSE, no detailed comparison is possible with his investigations. It seems likely, however, that the "insect adrenaline" which he isolated from mealworms consisted of a mixture of biologically active and inactive catechol amines, the bulk of whose physiological effect was attributable to the noradrenaline it contained.

The small amount of adrenaline in insects which has been established in this investigation also permits an explanation of GREGERMAN's and WALD's results. With an extraction technique closely resembling that employed in the present study those authors, who did not use any biological testing method, were unable to find adrenaline in extracts from mealworms. For each preparation they used 35 g larvae, which, with the technique used here, is a small amount for demonstration of adrenaline by paper chromatography. Moreover, the amount of adrenaline under these circumstances was too small to produce a color reaction with potassium ferricyanide; the substance could only be demonstrated by examination in ultraviolet light. GREGERMAN and WALD found, after paper chromatography using phenol saturated with water, an irregular spot whose color reaction to potassium ferricyanide suggested that contaminating substances had prevented complete separation. On further paper chromatography of an eluate from this spot they obtained two discrete spots, one of which showed a red and the other a blue color on treatment with potassium ferricyanide. By concentrating the extracts *in vacuo* until almost dry and extracting with anhydrous acetone, it was possible in the present investigation to obtain extracts having a sufficient degree of purity to ensure satisfactory separation by paper chromatography. GREGERMAN and WALD also sought by means of colorimetric determination to demonstrate adrenaline in their extracts. They used the method devised by EULER and HAMBERG (1950) which is based on the observation that adrenaline with iodine gives a red coloration at pH 4 due to the occurrence of iodo-adrenochrome, while

noradrenaline under similar conditions gives rise to iodo-noradrenochrome at pH 6. In so doing they found that no coloration appeared on 90 seconds oxidation of the extract at pH 4. This result does not conflict with my own finding that mealworms contain 0.02—0.06 µg adrenaline per g body weight. The demonstration of adrenaline by colorimetric determination would require an extract prepared with approximately 500 g larvae. GREGERMAN and WALD's observation of a red coloration corresponding to 2 µg adrenaline per g tissue following 3 minutes oxidation at pH 6 is interesting, and it is not inconsistent with the present results; namely, that mealworms contain 1.3—2.2 µg noradrenaline per g body weight. With the methods employed, they reached the conclusion that the spots demonstrated by paper chromatography were caused by two ortho-dihydroxyphenols, possibly 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenyllactic acid. On the basis of their data no detailed discussion of this finding is possible.

In view of the high content of dopamine in extracts from insects, the effect of that substance was studied on cat's blood pressure and fowl's rectal cæcum. By comparison with adrenaline and noradrenaline, dopamine had only a very slight sympathicomimetic effect, as earlier demonstrated e. g. by HOLTZ *et al.* (1942). In the present investigation the activity ratio of adrenaline to dopamine was 100—200:1 on cat's blood pressure and 900—1,500:1 on fowl's rectal cæcum. Because of the difficulty of determining exactly the dopamine content of insects, only an approximate estimate was possible regarding the interference produced by dopamine in the biological determination of adrenaline and noradrenaline. In so doing, the dopamine content of 10—15 µg per g tissue was found to correspond to about 10—15 per cent of the noradrenaline values and about 15—30 per cent of the adrenaline values. It is therefore questionable whether adrenaline values of $\gtrsim$ 0.01 µg per g tissue merely express the dopamine present in the extracts. However, it may be pointed out — with a reservation for amounts of adrenaline of this order of magnitude — that in paper chromatography of concentrated extracts from insects a spot apparently due to adrenaline was observed.

The occurrence of catechol amines in insects — especially the high content of noradrenaline and dopamine during the larval

stage and in the imagos, together with the inappreciable amount demonstrable in the early pupal stage — suggests that the use of insect material would throw light on those factors that control the production and decomposition as well as the functions of these amines. In higher vertebrates noradrenaline is the specific adrenergic neurotransmitter hormone, and this may also be the case in insects which have highly developed nervous systems. The high content of dopamine is more difficult to explain. Yet dopamine also occurs in higher animals; and although its functions are still unknown it has been suggested that it may constitute a precursor in the biosynthesis of noradrenaline, in which case its coexistence with that substance is explained. The high content of dopamine in insects — ten to twenty times greater than that of noradrenaline — is comparable with the relatively large amount demonstrated in healthy human urine (EULER, HAMBERG and HELLNER, 1951).

CHAPTER V

Catechol Amines in Lower Vertebrates and Their Effect on Isolated Heart

Earlier Investigations

The occurrence of chromaffin cells in fish has long been known and many investigations have been conducted in this field. GIACOMINI (1902 a, b, 1904, 1906), demonstrated chromaffin cells in *Cyclostomata, Teleostei, Ganoidei* and *Dipnoe;* and GASKELL (1912) verified his results in the lamprey *(Petromyzon fluviatilis).* GASKELL showed that extracts from chromaffin tissue of the lamprey, even of the sinus venosus region of the heart, produced a rise in blood pressure in the cat similar to that obtained with small doses of adrenaline. GIACOMINI's results in lung fishes were confirmed by HOLMES (1950), whose findings helped to elucidate the topography of the chromaffin cells in the African lungfish *(Proptopterus).*

LEYDIG (1852) described agglomerations of certain cells in *Elasmobranchi* — the so-called vascular glands or suprarenal bodies — which in his opinion were homologous with mammalian adrenals. According to BALFOUR (1878), the suprarenal bodies in this Group developed from the sympathetic ganglionic tissue. This finding was verified by KOHN (1903), who also investigated the occurrence of chromaffin cells in mammalian embryos. He found that they were formed during the embryonal development of the sympathetic ganglionic cells, and subsequently evolved into masses of chromaffin tissue — paraganglia — which were in more or less extensive communication with the sympathetic nervous system. The morphology and distribution of the suprarenal bodies and inter-renal organs (another type of the so-called vascular bodies) in *Elasmobranchi* have been investigated by GRYNFELTT (1902) and others.

POLL (1903) studied histologically the development of the inter-renal organs in the dogfish, and showed that they were homo-

logous with the adrenal cortex in mammals — a finding which is generally accepted today. ABOIM (1944), for instance, demonstrated that the inter-renal organ in the ray corresponded to the adrenal cortex of mammals.

The physiological significance of the suprarenal bodies and inter-renal organs of *Elasmobranchi* were investigated by VINCENT (1897), among others. He showed that extracts from suprarenal bodies produced a substantial pressor action, but whole extracts from inter-renal organs were only slightly active in the dog. BIEDL and WIESEL (1902) found that extracts from suprarenal bodies had the same vasoconstrictor effect as those from adrenals of higher animals. LUTZ and WYMAN (1927) studied extracts from inter-renal organs and suprarenal bodies by comparing their effects on the isolated frog eye. They found that the inter-renal organ extracts were inactive, but that the suprarenal bodies had a pronounced mydriatic effect.

Very few investigations have been reported on the occurrence and distribution of catechol amines in fish and batrachians. EULER (1952), who studied the occurrence of adrenaline and noradrenaline in *Elasmobranchi, Teleostei* and some invertebrates, found that chromaffin tissue from the two first-named contained those substances in varying proportions. Visceral organs (heart, liver, spleen) contained one-tenth to one-fiftieth of the amounts of noradrenaline found in corresponding organs from mammals. The adrenaline content was proportionally much higher than in mammals (40—60 per cent of the total catechols). SHEPHERD and WEST (1952) and SHEPHERD, WEST and ERSPAMER (1953) investigated, by chromatographic separation and biological testing, the occurrence of catechol amines in chromaffin tissue from various *Elasmobranchi.* They found substantial amounts of adrenaline and noradrenaline, the former substance constituting 20—34 per cent of the total catechols. SHEPHERD and WEST (1953) divided the chromaffin bodies from dogfish into three different parts, consisting of (1) those in the anterior segments represented by large chromaffin structures dorsal to the stomach, (2) smaller bodies in the middle segments, and (3) those lying adjacent to the inter-renal organ. They found adrenaline and noradrenaline in all preparations, the activity being greatest in extracts from chromaffin tissue adjacent to the inter-renal organ. In the ad-

renals of toads, noradrenaline has been demonstrated by HOUSSAY *et al.* (1950) and CABIB (1951).

In amphibians, LOEWI (1936) and EULER (1946 c) demonstrated adrenaline in extracts from frog's heart. In the secretion of the skin glands of toads adrenaline was first isolated by ABEL and MACHT (1912), and in various species LASAGNA (1951), LEE and CHEN (1951) and ÖSTLUND (unpublished observations, 1952) have found noradrenaline too. GREGERMAN (1952), using the paper chromatographic method, demonstrated both adrenaline and dopamine in the secretion of the skin glands of the toad *(Bufo marinus)*.

As regards the effect of catechol amines on isolated hearts of fish and invertebrates, that of adrenaline has already been studied by many investigators. WEST (1947) who studied the effect of adrenaline and noradrenaline on the isolated heart of frog found that the activity ratio of adrenaline to noradrenaline varied between 8—33:1 depending on the perfusion method used. Part of this literature will be referred to in this chapter, but for further details and references, *vide* FÄNGE and ÖSTLUND (1954).

Material

In the present investigation, the occurrence of catechol amines was studied in organs from the following species: hagfish *(Myxine glutinosa)*, dogfish *(Squalus acanthias)*, ray *(Raja batis)*, codfish *(Gadus callarias)*, frog *(Rana temporaria)* and toad *(Bufo vulgaris)*. Immediately after death the organs were removed and subjected to extraction, or placed in a deep-freeze for later preparation. Kidneys and inter-renal organs were dissected out and extracted separately. No attempts were made to remove the chromaffin cell tissue adjacent to those organs. The perfusion experiments were conducted on isolated hearts of fishes that had been kept in an aquarium with running sea water. Immediately after the animals had been killed, the hearts were carefully removed and placed in the perfusion apparatus (Fig. 2). Tyrode's solution was used for frog and toad hearts. The other perfusion solutions employed were mixtures of filtered sea water (approximately 3 per cent salinity) and distilled water, with the addition of 0.5 g glucose per liter. The following dilutions were used:

Two parts sea water and one part distilled water for hagfish.

One part sea water and one part distilled water for dogfish and ray.

One part sea water and two parts distilled water for codfish.

Undiluted sea water for squid.

Results

A. *Cyclostomata* Hagfish *(Myxine glutinosa)*

Extracts from gills and liver contained a hypotensive factor that made reliable determination of the adrenaline and noradrenaline content impossible. However, the hypertensive effect was only very slight with extracts representing 1—4 g tissue wet weight. On testing on fowl's rectal cæcum the low content of sympathicomimetic activity was verified. The total content of catechol amines in these organs was less than 0.01 μg per g tissue in two separate preparations. Extracts from heart contained 0.83 μg noradrenaline and 5.0 μg adrenaline per g wet weight, the adrenaline amounting to 86 per cent of the total catechols. (Fig. 19, Table 5.) Paper chromatography of heart extracts showed three spots (Fig. E) the positions of which were the same as those of adrenaline, noradrenaline and catechol-4 (see Chapter III).

The isolated heart of the hagfish was completely insensitive to varying doses of adrenaline, noradrenaline, dopamine, tyramine and acetylcholine, but reacted immediately to potassium chloride 2×10^{-4}. Nor, after perfusion with physostigmine 1×10^{-6} for five minutes, was any effect obtained with acetylcholine (Fig. 24).[1]

B. *Elasmobranchi* 1. Dogfish *(Squalus acanthias)*

Spleen. — Two extracts prepared immediately after death contained, respectively, 0.085 μg and 0.032 μg noradrenaline and 0.022 μg adrenaline per g tissue wet weight; whereas an extract prepared 4—12 hours after death contained 0.014 μg noradrenaline and $<$ 0.01 μg adrenaline per g tissue.

Kidneys and inter-renal organs. — Kidneys from which the inter-renal organs had not been removed contained 0.93 μg noradrenaline and 0.13 μg adrenaline per g tissue, probably due to the presence of chromaffin cells. Two extracts from inter-renal organs contained 47 and 57 μg noradrenaline and 6 and 10 μg adrenaline per g tissue. During preparation of the inter-renal

[1] Figures 24—29 see pages 56—59.

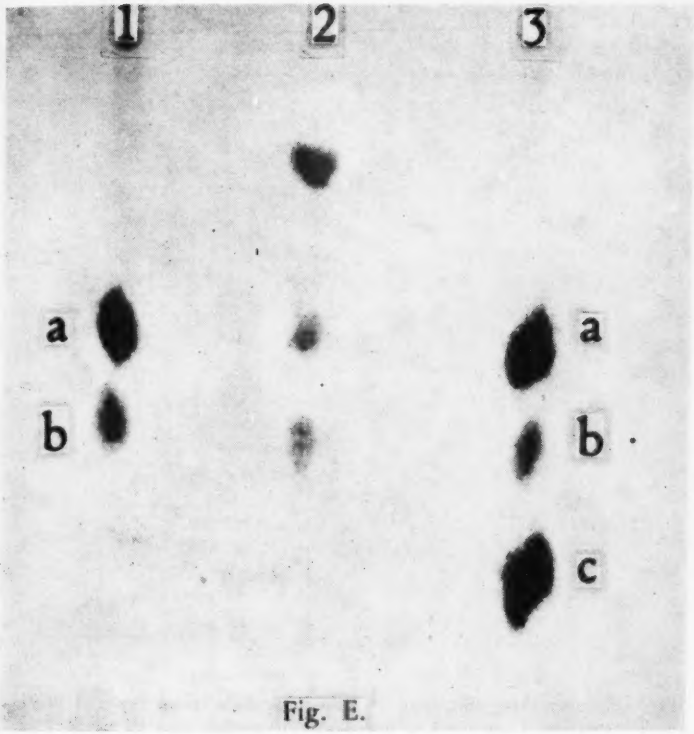


Fig. E.

Fig. E. Paper chromatography of purified extract from hagfish heart. Solvent n-butanol saturated with N HCl. Descending time 17 hours. Sprayed with 0.1 per cent ninhydrin and 0.44 per cent potassium ferricyanide.
1 and 3. Standard solutions of noradrenaline (a), adrenaline (b), and dopamine (c).
2. Purified extract from hagfish heart corresponding to 15 g tissue.

organs the adjacent tissue was included, so that the presence of chromaffin tissue could not be ruled out. Paper chromatography of inter-renal tissue demonstrated the presence of considerable amounts of noradrenaline, smaller quantities of adrenaline, and of catechol-4 (Figs. 12, 14 and F, Table 4).

Heart. — Four extracts from dogfish hearts contained, respectively, 0.022, 0.030, 0.035 and 0.11 µg noradrenaline, and

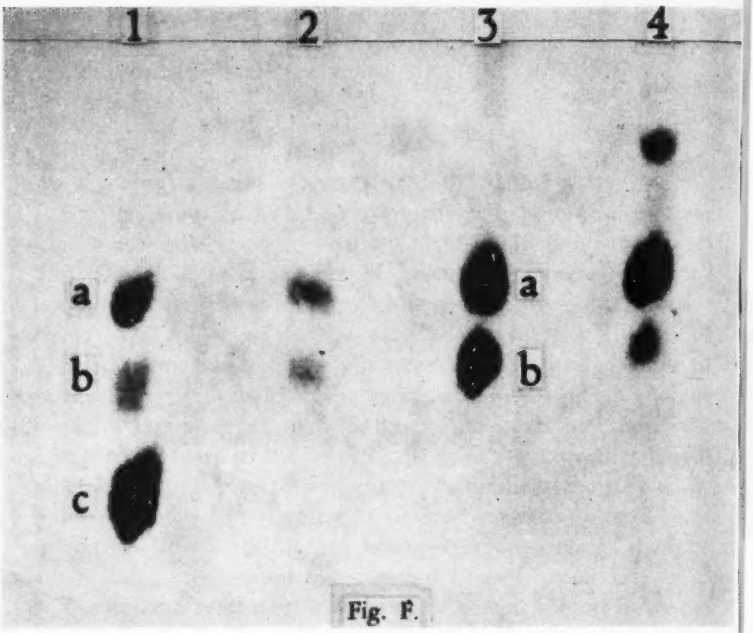


Fig. F.

Fig. F. Paper chromatography of inter-renal tissue from ray and dogfish. Solvent n-butanol saturated with N HCl. Descending time 15 hours. Photograph taken after spraying with 0.1 per cent ninhydrin and 0.44 per cent potassium ferricyanide.

1 and 3. Standard solutions of noradrenaline (a), adrenaline (b), and dopamine (c).

2. Purified extract from inter-renal organ of ray corresponding to 0.5 g tissue.

4. Purified extract from inter-renal organ of dogfish corresponding to 7 g tissue.

0.021, 0.043, 0.028 and 0.032 µg adrenaline per g tissue. The lowest contents of catechol amines were found in the extracts prepared more than 6 hours after death (Table 5).

Heart perfusion. — The isolated heart of the dogfish showed varying sensitivity to catechol amines. Both adrenaline and noradrenaline had positive inotropic and chronotropic effects

Table 4

Adrenaline and Noradrenaline Content in Visceral Organs of Fishes and Frogs. Trichloroacetic Acid Extraction. Biological Assay on Cat's Blood Pressure and Fowl's Rectal Cæcum. All Figures Refer to Wet Weight. (*Cf.* Figs. 16, 18, 20.)

Species	Number of Animals	Organ	Total Weight of Tissue in G	Noradrenaline µg/g	Adrenaline µg/g	Adrenaline %
Dogfish	23	Spleen	140	0.085	0.022	21
»	15	»	140	0.014	<0.010	—[1]
»	5	»	50	0.032	0.022	41
»	10	Kidney and inter-renal	200	0.93	0.13	12
»	4	Inter-renal and adjacent tissue	2	47	6.0	11
»	10	»	6	57	10	15
Ray	8	Spleen	50	0.042	0.024	36[1]
»	—	»	56	0.42	0.35	46
»	—	Kidney and inter-renal	53	0.70	1.7	71
»	—	Inter-renal	0.16	100	95	49
»	—	Kidney	100	0.29	0.027	9
Codfish	7	Spleen	26	0.023	0.034	60[1]
»	24	»	11	0.040	0.12	75
Frog	272	Spleen	4.0	0.52	0.38	42
»	203	»	3.0	0.75	0.21	22
»	9	Liver	6.7	0.032	0.038	54
»	7	»	6.8	0.040	0.038	49
»	90	»	94	0.032	0.048	60

(Fig. 27). In some experiments adrenaline and noradrenaline had an initial inhibitory effect similar to that of acetylcholine and which, like the latter, could be blocked with atropine (Fig. 28). In physiological doses tyramine and dopamine had only a very slight sympathicomimetic effect or none at all. The threshold value for adrenaline was 1×10^{-9}, and the activity ratio of adrenaline to noradrenaline was 50—100:1.

[1] Preparation made 4—12 hours after death.

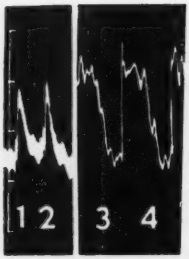


Fig. 12.

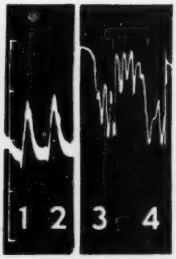


Fig. 13.

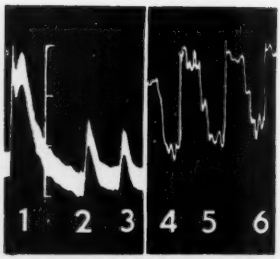


Fig. 14.

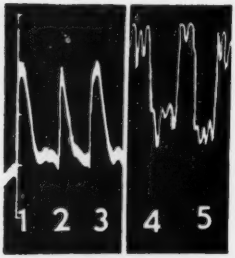


Fig. 15.

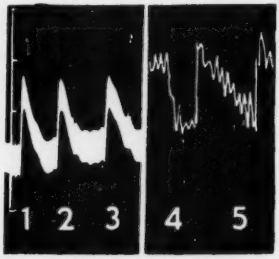


Fig. 16.

Fig. 17.

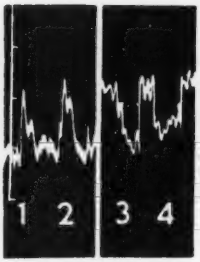


Fig. 18.

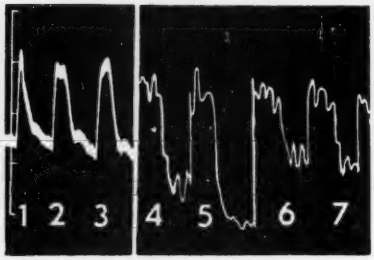


Fig. 19.

2. Ray *(Raja batis)*

Spleen. — Deep-frozen fresh tissue contained 0.42 μg noradrenaline and 0.35 μg adrenaline, the corresponding contents in tissue 4—12 hours after death being 0.042 μg and 0.024 μg per g respectively.

Kidneys and inter-renal organs. — Prepared together kidney and inter-renal contained 0.70 μg noradrenaline and 1.7 μg adrenaline per g; inter-renal tissue that had been dissected free contained

Figs. 12—19. Biological assay on cat's blood pressure and fowl's rectal cæcum of extracts from some organs in fishes. (*Cf.* Tables 4 and 5). Blood pressure ordinate 80—160 mm Hg.

Fig. 12. Dogfish spleen. q = 0.65; Q = 25.
1. Extract corresponding to 0.5 g tissue.
2. 0.05 µg noradrenaline.
3. Extract corresponding to 0.4 g tissue.
4. 0.25 µg noradrenaline.

Fig. 13. Dogfish heart. q = 0.5; Q = 20.
1. Extract corresponding to 1 g tissue.
2. 0.15 µg noradrenaline.
3. 0.15 µg noradrenaline.
4. Extract corresponding to 0.25 g tissue.

Fig. 14. Dogfish kidney and inter-renal. q = 0.5; Q = 28.
1. Extract corresponding to 0.25 g tissue.
2. Extract corresponding to 0.06 g tissue.
3. 0.05 µg noradrenaline.
4. Extract corresponding to 0.06 g tissue.
5. 0.25 µg noradrenaline.
6. Extract corresponding to 0.05 g tissue.

Fig. 15. Ray spleen. q = 0.5; Q = 20.
1. Extract corresponding to 0.5 g tissue.
2. 0.3 µg noradrenaline.
3. 0.6 µg adrenaline.
4. Extract corresponding to 0.04 g tissue.
5. 0.3 µg noradrenaline.

Fig. 16. Ray inter-renal. q = 0.5; Q = 20.
1. 0.15 µg noradrenaline.
2. Extract corresponding to 0.001 g tissue.
3. 0.3 µg adrenaline.
4. Extract corresponding to 0.00005 g tissue.
5. 0.1 µg noradrenaline.

Fig. 17. Codfish heart. q = 0.6; Q = 20.
1. Extract corresponding to 2 g tissue.
2. 0.05 µg noradrenaline.
3. Extract corresponding to 0.45 g tissue.
4. 0.2 µg noradrenaline.

Fig. 18. Codfish spleen. q = 0.5; Q = 28.
1. Extract corresponding to 2.5 g tissue.
2. 0.1 µg noradrenaline.
3. Extract corresponding to 0.25 g tissue.
4. 0.2 µg noradrenaline.

Fig. 19. Hagfish heart. q = 0.5; Q = 25.
1. 0.25 µg noradrenaline.
2. Extract corresponding to 0.07 g tissue.
3. 0.5 µg adrenaline.
4. 0.15 µg noradrenaline.
5. Extract corresponding to 0.0015 g tissue.
6. Extract corresponding to 0.00075 g tissue.
7. 0.1 µg noradrenaline.

100 µg noradrenaline and 95 µg adrenaline per g tissue. (Figs. 15 and 16, Table 4.) No attempt was made to rule out the possible presence of adjacent chromaffin tissue. Paper chromatography of inter-renal organs showed two spots in the positions of adrenaline and noradrenaline (Fig. F).

Table 5

Adrenaline and Noradrenaline Content in Heart Tissue of Fishes and Frogs. Trichloroacetic Acid Extraction. Biological Assay on Cat's Blood Pressure and Fowl's Rectal Cæcum. All Figures Refer to Wet Weight. (*Cf.* Figs. 13, 17, 19, 21.)

Species	Number of Animals	Total Weight of Heart Tissue in G	Noradrenaline µg/g	Adrenaline µg/g	Adrenaline %
Hagfish	120	14	0.83	5.0	86
Dogfish	23	60	0.035	0.028	44
»	15	40	0.030	0.043	59[1]
»	36	100	0.022	0.021	49
»	12	34	0.11	0.032	2?
Ray	8	48	0.087	0.025	22[1]
Codfish	24	10	0.15	0.10	40
»	40	120	0.014	0.011	44
»	9	39	0.015	0.022	59
Frog	272	57	0.34	0.92	73
»	203	46	0.10	1.0	91
»	40	6.5	0.14	0.76	84

Heart. — An extract of heart contained 0.087 µg noradrenaline and 0.025 µg adrenaline per g tissue.

Heart perfusion. — The isolated heart of the ray, like that of the dogfish, reacted to adrenaline and noradrenaline with positive inotropic and chronotropic effects. Ray heart was in general less sensitive to catechol amines than was dogfish heart. The threshold value was 1×10^{-8} for adrenaline and 1×10^{-7} for noradrenaline, and the activity ratio of adrenaline to noradrenaline was 4—10:1. In some experiments adrenaline and noradrenaline showed an initial inhibitory effect which, as in the case of dogfish heart, was blocked by atropine. Tyramine and dopamine in physiologicai doses had only a very slight sympathicomimetic effect or none at all.

[1] Preparation made 4—12 hours after death.

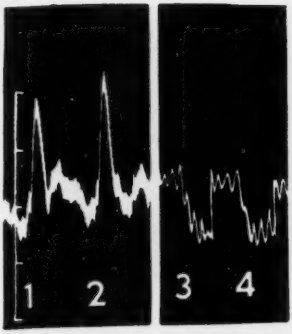
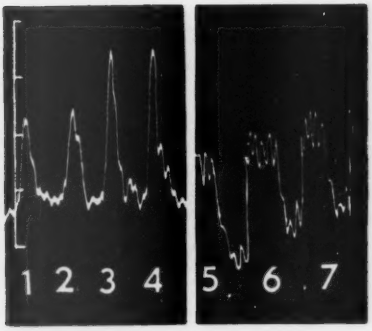


Fig. 20.



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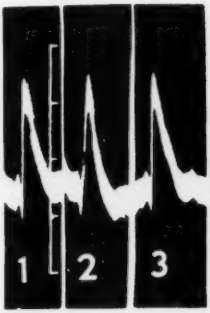

Fig. 22.

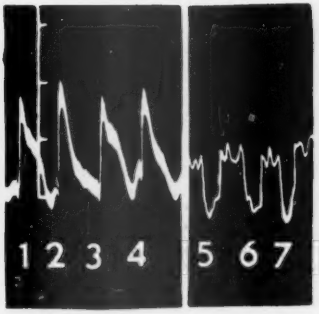

Fig. 23.

Figs. 20—23. Biological assay on cat's blood pressure and fowl's rectal cæcum of extracts from some organs in frog and toad (*Cf.* Tables 4 and 5). Blood pressure ordinate 80—160 mm Hg.

Fig. 20. Frog spleen. q = 0.65; Q = 25.
1. Extract corresponding to 0.1 g tissue.
2. 0.1 µg noradrenaline.
3. 0.3 µg noradrenaline.
4. Extract corresponding to 0.03 g tissue.

Fig. 21. Frog heart. q = 0.5; Q = 27.
1. 0.1 µg noradrenaline.
2. 0.2 µg adrenaline.
3. Extract corresponding to 0.25 g tissue.
4. 0.2 µg noradrenaline.
5. 0.3 µg noradrenaline.
6. Extract corresponding to 0.01 g tissue.
7. 0.2 µg noradrenaline.

Fig. 22. Frog adrenal.
1. 2 µg adrenaline.
2. 0.25 µg noradrenaline.
3. Extract corresponding to 0.005 g tissue.

Fig. 23. Toad *(Bufo vulgaris)* skin glands. q = 0.6; Q = 30.
1. 0.5 μg adrenaline.
2. 0.35 μg noradrenaline.
3. Extract corresponding to 0.1 g tissue.
4. 0.3 μg noradrenaline.
5. Extract corresponding to 0.005 g tissue.
6. 0.5 μg noradrenaline.
7. Extract corresponding to 0.007 g tissue.

C. *Teleostei* Codfish *(Gadus callarias)*

Spleen. — An extract from 24 small specimens contained 0.040 μg noradrenaline and 0.12 μg adrenaline per g, but a similar extract from fish 4—12 hours after death had a content of 0.023 μg and 0.034 μg respectively per g tissue (Fig. 18, Table 4).

Heart. — An extract from 24 small specimens contained 0.15 μg noradrenaline and 0.10 μg adrenaline per g tissue (Fig. 17, Table 5).

Heart perfusion. — On the isolated codfish heart adrenaline and noradrenaline had a slightly positive inotropic and chronotropic effect. The threshold value for adrenaline was 1×10^{-7}, and the activity ratio of adrenaline to noradrenaline 10—20:1 (Fig. 29).

Dopamine and tyramine in physiological doses had no demonstrable effect on the isolated heart of the codfish.

D. *Amphibia* Frog *(Rana temporaria)* and toad *(Bufo vulgaris)*

Heart. — Three heart extracts from frogs contained, respectively, 0.10, 0.14 and 0.34 μg noradrenaline and 1.0, 0.76 and 0.92 μg adrenaline per g tissue, the adrenaline thus amounting to 75—91 per cent of the total catechol amines (Fig. 21, Table 5). The presence of adrenaline and noradrenaline was demonstrated by paper chromatography. Noradrenaline was present in too small a quantity to be detected in ordinary light, but was demonstrable in ultraviolet light (Fig. G).

Spleen. — Two extracts from spleen contained, respectively, 0.75 μg and 0.52 μg noradrenaline and 0.21 and 0.38 μg adrenaline per g tissue, the adrenaline amounting to 22—42 per cent of the total catechol amines (Fig. 20, Table 5).

Liver. — Three liver extracts contained 0.032, 0.040 and 0.032 μg noradrenaline and 0.038, 0.038 and 0.048 μg adrenaline

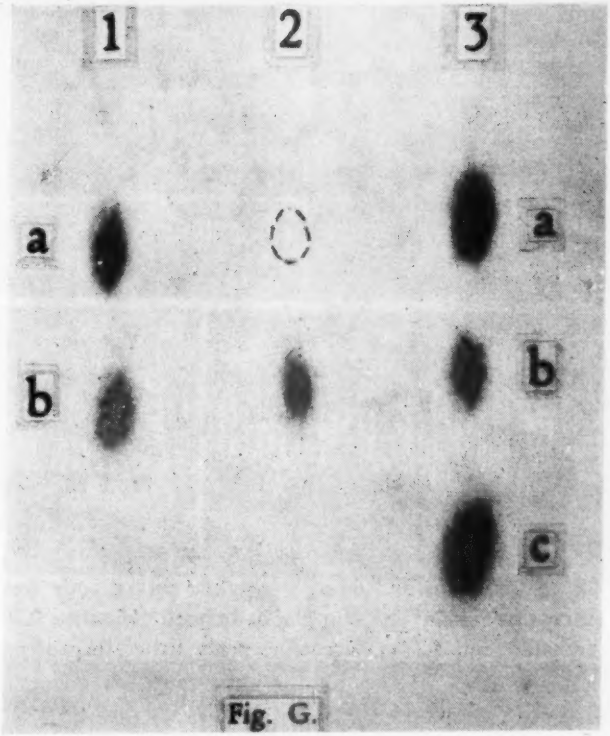

Fig. G. Paper chromatography of purified extract from heart tissue of frog. Solvent n-butanol saturated with N HCl. Descending time 22 hours. Photograph taken after spraying with 0.1 per cent ninhydrin and 0.44 per cent potassium ferricyanide.

1 and 3. Standard solutions of noradrenaline (a), adrenaline (b), and dopamine (c).

2. Heart of the frog. Extract corrresponding to 75 g tissue. (The dotted area indicates a spot observed in ultraviolet light.)

respectively per g tissue, corresponding to about 50 per cent adrenaline of the total catechol amines.

Adrenal. — Kidneys and adrenals together contained 244—270 µg noradrenaline and 112—153 µg adrenaline per kg body weight (Fig. 22).

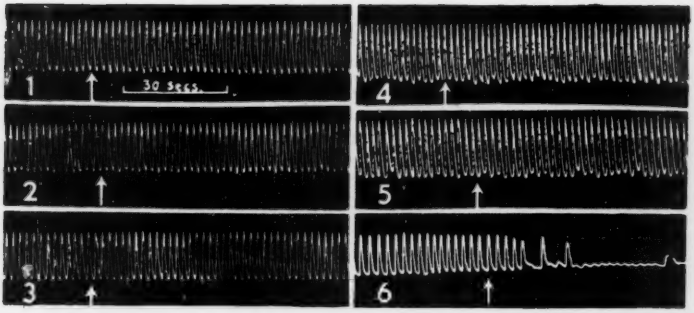

Fig. 24.

Fig. 24. Hagfish heart perfusion *in vitro.*

1. Acetylcholine	1×10^{-7}	3. Acetylcholine	5×10^{-6}
2. Acetylcholine	5×10^{-7}		

Between perfusions 1 and 2 the heart was perfused with physostigmine 1×10^{-6} for five minutes.

4. Adrenaline	5×10^{-7}	6. Potassium chloride	2×10^{-4}
5. Noradrenaline	5×10^{-7}		

A corresponding preparation from toad showed contents of 613 μg noradrenaline and 83 μg adrenaline per kg body weight. An extract from skin and skin glands of toad contained 0.20 μg noradrenaline and 3.7 μg adrenaline per g tissue, the adrenaline amounting to 95 per cent of the total catechol amines (Fig. 23).

Adrenaline and noradrenaline were identified by paper chromatography of extracts from adrenal tissue dissected out of frogs and toads.

Heart perfusion. — Adrenaline and noradrenaline had positive inotropic and chronotropic effects on isolated heart of frog. The activity ratio of adrenaline to noradrenaline was 10—20:1.

E. *Mollusca* Squid *(Eledone cirrosa)*

Heart perfusion. — The squid heart showed a high and equal sensitivity to adrenaline and noradrenaline in the form of a strongly positive inotropic and a moderately positive chronotropic reaction. The threshold value was 1×10^{-10} for both adrenaline and noradrenaline. Tyramine and dopamine in physiological doses had no effect on this heart (Fig. 25).

Repeated doses of adrenaline and noradrenaline had a desensitizing effect on the heart (Fig. 26). An interval of 5—10 minutes between tests was sufficient to abolish the effect.

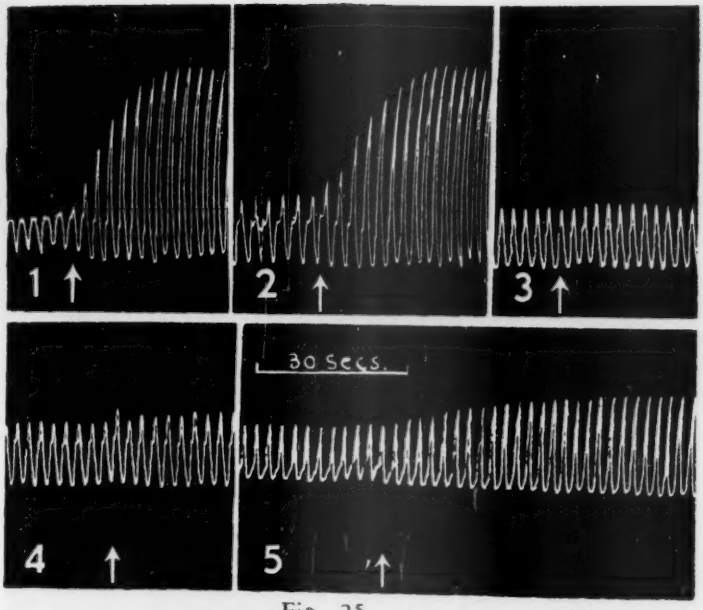

Fig. 25.

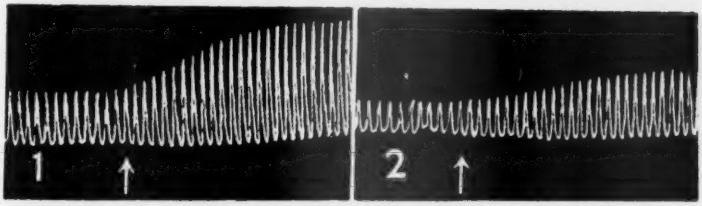

Fig. 26.

Fig. 25. Squid heart perfusion *in vitro*.

1. Adrenaline	1×10^{-8}	4. Tyramine	4×10^{-8}
2. Noradrenaline	1×10^{-8}	5. Dihydroergotamine	2×10^{-5}
3. Dopamine	4×10^{-8}		

Fig. 26. Squid heart perfusion *in vitro*.

1. Adrenaline	2×10^{-7}	2. Adrenaline	2×10^{-7}

Perfusion 2, which commenced immediately after normalization of the heart, shows the desensitizing effect of adrenaline in repeated perfusions without intervals.

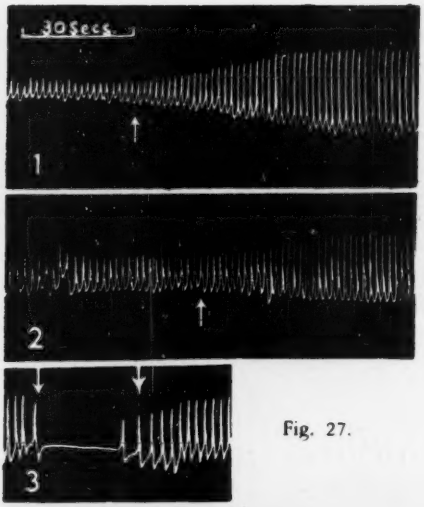

Fig. 27.

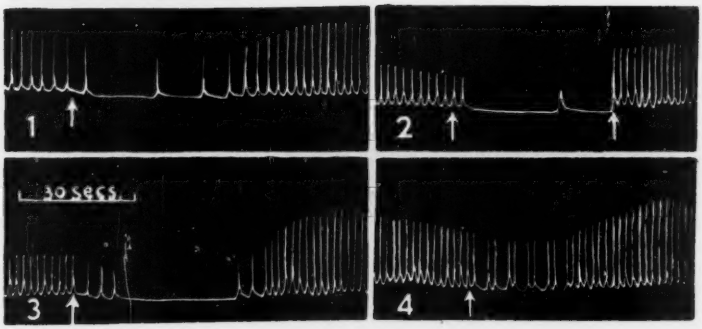

Fig. 28.

Fig. 27. Dogfish heart perfusion *in vitro.*

1. Adrenaline 1×10^{-8} 3. Acetylcholine 5×10^{-7}
2. Noradrenaline 5×10^{-7}

Between perfusions 2 and 3 the heart was perfused with physostigmine 4×10^{-5} for three minutes. Before treatment with physostigmine this heart was insensitive to acetylcholine 1×10^{-6} perfusion.

Fig. 28. Dogfish heart perfusion *in vitro.*

The initial inhibitory effect of catechol amines.

1. Noradrenaline 1×10^{-8} 3. Adrenaline 5×10^{-9}
2. Acetylcholine 5×10^{-8} 4. Adrenaline 5×10^{-9}

Between 3 and 4, perfusion with atropine 1×10^{-5} for three minutes.

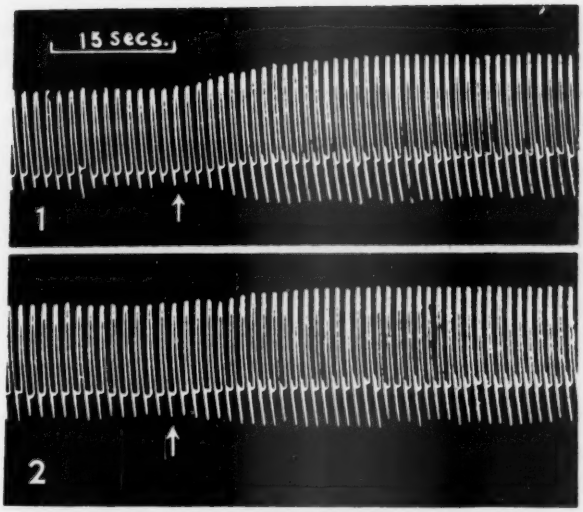

Fig. 29.

Fig. 29. Codfish heart perfusion *in vitro.*
1. Adrenaline 6×10^{-7}
2. Noradrenaline 1×10^{-6}

Discussion

The high content of catechol amines in the heart of the hagfish — the most primitive of recent vertebrates — is interesting. The catechol content of 4—6 μg per g tissue is twenty to one hundred times greater than that found in some *Elasmobranchi* and *Teleostei,* and four to five times greater than that in some mammals.

GIACOMINI, who studied the occurrence of chromaffin cells in *Cyclostomata,* including the lamprey *(Petromyzon marinus),* found chromaffin tissue round the vessels entering the heart. The results obtained in the present investigation suggest that tissue containing abundant catechol amines is present in or adjacent to the hagfish heart. This finding may be attributable to the occurrence of chromaffin cells either alone or together with postganglionic sympathetic nerve fibers. However, no such fibers have been demonstrated in *Cyclostomata.* Further studies, includ-

ing histologic investigations, are necessary to establish whether the catechol amines found are produced in the heart itself or in the adjacent tissue.

A noteworthy consideration is that the hagfish heart does not react to adrenaline and noradrenaline. Perfusion of isolated heart by adrenaline, noradrenaline and acetylcholine in increasing doses had no effect whatsoever. This finding accords with observations by FÄNGE (1952), who also experimented on the hagfish heart *in situ*. Heart prepared in this way did not react to adrenaline and acetylcholine. Stimulation of the vagus had no effect on the heart, but the gills, which are innervated by that nerve, did react. In this fish the vagus, in its course towards the intestinal organs, runs in close proximity to the heart, but whether the latter is innervated is still obscure. The heart nevertheless is rather differentiated, with a sinus venosus, atrium and ventricle so that nervous regulation is not improbable. However, the circulatory system of the hagfish presents considerable dissimilarities from those of higher vertebrates. Besides the ordinary heart *(cor venosum branchiale)*, this fish has a further three pulsatory organs; namely, a portal vein heart and two caudal hearts.

The total content of catechol amines in visceral organs (heart, spleen) of *Elasmobranchi* and *Teleostei* was consistent with the values obtained by EULER in his investigations on the occurrence of adrenaline and noradrenaline in marine and freshwater fish. The presence of catechol amines in the visceral organs of fish indicates that those organs have chromaffin cells or an adrenergic innervation. The effect of adrenaline and noradrenaline on isolated hearts from dogfish, ray and codfish is similar in some respects to that produced by those substances on the innervated hearts of higher animals. The effect of acetylcholine, too resembles that observed in higher animals. It should be pointed out here that acetylcholine sometimes has an initial inhibitory effect followed by a positive inotropic and chronotropic effect. In higher animals this adrenaline-like effect of acetylcholine has been studied by MCDOWALL (1944) and HOFFMANN *et al.* (1945).

MCDOWALL, in experiments on the isolated heart of the cat, found that acetylcholine in concentrations of 1×10^{-6} and higher caused slowing of the heart followed by acceleration. Both of these effects were blocked by atropine, which in small doses chiefly

inhibited the decelerating effect, whereas the accelerating effect could be abolished by ergotoxine. The same author obtained similar results on isolated hearts from rabbits, frogs and rats.

HOFFMANN *et al.* found that injection of acetylcholine into isolated atropinized hearts of dogs, cats, rabbits and guinea pigs regularly caused an adrenaline-like effect, *i. e.*, an increase of heart rate and amplitude, as well as a change of blood flow in the coronary vessels. This effect was blocked by ergotamine, curare and nicotine. In perfusate from isolated hearts they were able to demonstrate biologically an adrenaline-like substance that was liberated on injection of acetylcholine or nicotine. They wrote: "We are unable to decide whether, in the case of the heart, we deal with ganglionic sympathetic structures, with chromaffin tissue, or with a combination of both".

A noteworthy observation is that adrenaline in adequate doses occasionally has an initial acetylcholine-like effect on the isolated heart. This was observed in sharks by MACDONALD (1925) and LUTZ (1930), and in toads by LUTZ (1933). In the present investigation this initial effect of adrenaline was obtained on some isolated hearts of rays and sharks; and noradrenaline had a similar effect. By perfusing such hearts with atropine it was possible to block this effect. This observation would be explained if acetylcholine-producing structures, stimulated by adrenaline and noradrenaline, were present locally in the heart. However, further investigations — for instance, acetylcholine determinations in heart perfusate before and during administration of catechols — are required to establish whether this is the case.

The occurrence of catechol amines in the kidneys of *Elasmobranchi* was probably due to chromaffin tissue having adhered to those organs. The presence of considerable amounts of catechols in extracts from inter-renal organs from those fish may have a similar explanation. In this connection, however, it is important to bear in mind the following: On comparison of the catechol amine contents of kidneys and inter-renal organs, an accumulation of activity in or adjacent to the latter was found. In the ray the inter-renal organs are scattered in the form of small discrete areas on the surface of the kidney, whereas in the shark the single organ is located between the kidneys.

The results obtained show that an accumulation of sympathicomimetic activity is present in the immediate vicinity of these inter-renal organs. No attempts were made to rule out that chromaffin tissue located there was the sole source of the biological activity.

During the embryonal development of the adrenals in higher vertebrates, the adrenal medulla consists of chromaffin cell masses located outside the cortical tissue; but in the adult animal it is enclosed by the latter. Analogously, a mass of chromaffin tissue adjacent to the inter-renal organ in *Elasmobranchi* might be chiefly comparable with the adrenal medulla in the higher animal, even though the separation is still total.

Tests on cat's blood pressure served to confirm earlier observations that the sympathicomimetic activity in extracts from frog heart (LOEWI, EULER) was mainly to be attributed to adrenaline. Biological assay on cat's blood pressure and fowl's rectal cæcum, as well as paper chromatography, showed, however, that small amounts of noradrenaline too were present in extracts from frog heart, the amounts corresponding to 10—20 per cent of the total catechols.

In those perfusion experiments that were conducted on isolated hearts of frogs and toads, adrenaline and noradrenaline were not observed to have any initial inhibitory effect. That such an effect occurs at times has been shown, however, by LUTZ and others.

CHAPTER VI

General summary

The procedures used for extraction, purification, estimation and identification of noradrenaline, adrenaline and dopamine in extracts from various animal species are described. (Chapter II)

In the ganglionic chain of the earthworm adrenaline and noradrenaline were found in amounts of 1.4 μg and 0.32 μg respectively per g tissue wet weight.

No conclusive evidence was obtained of the occurrence of adrenaline and noradrenaline in a number of species representing *Protozoa, Coelenterata, Echinoderma, Crustacea,* and *Tunicata.*

Paper partition chromatography of extracts from *Noctiluca,* sea anemone, and the common mussel showed the presence of a substance having an R_f value of 0.02—0.03 when using phenol saturated with 0.1 N HCl as a solvent. The chemical and biological properties agreed with those of a catechol amine. The spot could be differentiated from adrenaline, noradrenaline, dopamine, dopa, tyrosine and 5-hydroxytryptamine. For purposes of reference it was named "catechol-4". (Chapter III)

All of the insects examined contained catechol amines in considerable quantities. In the worker bee the contents of noradrenaline and dopamine were highest in larvae and adult insects, being low in the early and higher in the late pupal stage. Mealworms contained more noradrenaline and dopamine per g tissue in the posterior portion, and more adrenaline in the anterior portion. Catechol-4 was also found in the anterior portions of mealworms. (Chapter IV)

The heart of the hagfish *(Myxine glutinosa)* contained a remarkably high content of adrenaline and noradrenaline (5—6 μg per g tissue). It also contained catechol-4. The liver and gills of the hagfish contained small amounts of sympathicomimetic activity together with a hypotensive factor.

Liver, spleen and heart of *Elasmobranchi* and *Teleostei* contained small amounts of adrenaline and noradrenaline. Extracts from kidneys and inter-renal organs contained considerable amounts of catechol amines, presumably due to adjacent chromaffin tissue. Here, too, catechol-4 was demonstrated. (Chapter V)

Liver and spleen of frog *(Rana temporaria)* contained adrenaline and noradrenaline. The heart contained about 1 μg catechol amines per g tissue, of which adrenaline made up about 90 per cent.

The effect of adrenaline and noradrenaline was studied on isolated perfused hearts from hagfish, dogfish, ray, codfish and frog.

The hagfish heart was totally insensitive to adrenaline, noradrenaline and acetylcholine up to concentrations of 1×10^{-6}.

Hearts from dogfish, ray, codfish and frog reacted to adrenaline and noradrenaline with positive inotropic and chronotropic effects. The threshold concentrations were approximately 1×10^{-9} for adrenaline in dogfish, 1×10^{-8} in ray, 1×10^{-7} in codfish and 1×10^{-9} in frog. The activity ratio of adrenaline to noradrenaline varied in hearts from the same species and averaged 50—100:1 in the dogfish, 4—10:1 in the ray, 10—20:1 in the codfish, and 10—20:1 in the frog. Adrenaline and noradrenaline sometimes had an initial inhibitory effect on isolated hearts from dogfish and ray. This effect, which was similar to that obtained with acetylcholine, could be blocked by atropine. In all of the hearts investigated, dopamine and tyramine in physiological doses had only a very slight sympathicomimetic effect or none at all. (Chapter V)

The heart of the squid *(Eledone cirrosa)* showed a remarkable sensitivity to adrenaline and noradrenaline, responding in concentrations down to 1×10^{-10}. The activity ratio of adrenaline to noradrenaline was 1:1, and the heart reacted to these catechol amines with a pronounced inotropic and a moderate chronotropic effect. Dopamine and tyramine had a very slight sympathicomimetic effect or none at all. (Chapter V)

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